RESEARCH ARTICLE

Heavy metal concentrations in *Mullus barbatus* and *Pagellus erythrinus* in relation to body size, gender, and seasonality

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Abstract Marine environments have been subjected to an increase in heavy metal pollution. Investigations were conducted in the bioaccumulation of heavy metals for both a benthic (*Mullus barbatus*) and a benthopelagic fish species (*Pagellus erythrinus*). The aim of this study was to examine the concentration levels of four metals in the body tissue of two fish species, in Pagasitikos Gulf in Greece, and to determine if metal concentration levels were affected by season, size, and species. Fish samples were collected monthly from September 2009 to August 2010. Chromium (Cr), Copper (Cu), Zinc (Zn), and Cadmium (Cd) concentrations were measured in muscle, gills, vertebral column, and in the "remaining fish sample." Statistical analysis pinpointed substantial differences in metal concentration levels between some size classes. Significant differences were observed between two fish species' tissues concerning Cu, Zn, and Cd concentrations. Cu and Zn concentrations varied amongst red mullet tissues as did Zn and Cd concentrations in common pandora. Ample variations were found seasonally in metal concentration levels; however, nonsignificant statistical differences were found among sexes.

Keywords *Mullus barbatus* · *Pagellus erythrinus* · Heavy metals · Pagasitikos Gulf (Greece) · Size classes · Organ distribution · Bioaccumulation

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Introduction

Metals exist in nature as biochemical cycles supply sediments to marine environments with new intake of metals through biotic and abiotic processes. Urbanization, industrial development, agriculture, biospheric overload, and domestic sewage are the major anthropogenic reasons for the increasing pollution. These amplify the presence of increased limits of heavy metals (Alhas et al. 2009).

Metals play an important role in marine ecosystems and human consumption of fish, resulting in an increased interest for studying metal concentration in fish. There is an increasing concern in heavy metals' biomagnification owing to their simplified uptake through the food chain (Jaric et al. 2011). Metals may be transferred within the food chain affecting both aquatic and human organisms. This occurs both horizontally within the same trophic level and vertically among different trophic levels (Jara-Martini et al. 2009). The effects of metal toxicity ranges from reproductive issues, growth, and mortality to the total loss of some populations (Alhas et al. 2009). Toxicant exposure may have adverse effects on immigration, respiration, feeding, and reproduction (Atchison et al. 1987).

The city of Volos, its port and industrial area, result in the Pagasitikos Gulf having toxicological significance of anthropogenic contaminants. Food production, packaging facilities, wood units, and metal processing plants, including a cement plant, are the area's predominant activities (Tsangaris et al. 2013). The metals that have been examined in this study are used in industrial activities and also exist in the background shale of the Gulf (Salomons and Forstner 1984). Copper (Cu), chromium (Cr), and zinc (Zn) are metals related to anthropogenic activities (Catsiki and Strogyloudi 1999). Elements such as Zn and Cu play an important role in an organism's physiological process, but their accumulation can

disturb its biochemical processes (Ural et al. 2012). Cr is a fundamental element, but its toxicity is related to gill histology and hematology changes (Reid 2012). Zn is another vital element for life, but its toxicity depends on water chemistry (Hogstrand 2012). Cadmium (Cd) has high toxicity. Cd reacts with particular substances such as iron oxide and clay minerals within the aquatic environment. Its reaction may enter the food chain through the water column or sediment (McGeer et al. 2012).

The examination of different fish tissues and metal levels allows for greater understanding of the distribution of metal concentration in fish. Metal concentration levels were examined in specific fish organs and bones since they provide important information in complex fish bioaccumulation pathways. These parameters that are affected by heavy metal pollution emphasize the importance of these contaminants in the total assessment of the environmental stress (Catsiki and Strogyloudi 1999).

Throughout this study, concentrations of four metal levels were examined in two fish species (one demersal and one pelagic). In particular Cr, Cu, Zn, and Cd concentrations measured in gills, muscle, vertebral column, and the "remaining sample," in both *Mullus barbatus* and *Pagellus erythrinus*. Two fish species living in different column levels of the same environment were examined. Observations and examinations were conducted within species, specifically, during variations in seasons (sampling period), size class, gender, or maturity levels.

Materials and methods

Study area

Metal concentrations were studied in sediment taken from Pagasitikos Gulf in the past (e.g., Voutsinou-Taliadouri and Satsmadjis 1982; Voutsinou-Taliadouri et al. 2007), while the latest published study was in sediments and transplanted mussels (Tsangaris et al. 2013). Two common and commercial fish species, the demersal fish *M. barbatus* and the benthopelagic *P. erythrinus*, were collected from the Pagasitikos Gulf—a shallow semi-enclosed gulf in the western Aegean Sea. Sampling depth varied from 21 to 54.25 m. The used trammel net contained 19–32 mm meshes. The sampling area is shown in Fig. 1.

Sample collection and preparation

Fish samples were collected on a monthly basis starting from September 2009 to August 2010. All samples were coded and measured for total body weight (W, in grams), gonad weight (Wg), liver weight (Wl), and total length (TL, in centimeters) (precision, ±0.01 cm); gonadosomatic index (GSI=100*Wg/W), hepatosomatic index (HSI=100*Wl/W), and condition factor (K=100*W/L^b) were also calculated.

Their total length was used to classify individuals in size classes for each species. Fish were classified into three size classes (I: small, II: medium, III: large). Particularly, red mullet was divided into the following three size classes: I (11.2–16.2 cm), II (16.3–23.0 cm), and III (23.1–28.1 cm). The three size classes for common pandora were as follows: I (10.9–15.6 cm), II (15.7–22.0 cm), III (22.1–26.8 cm).

Whole gill and a 2–5 g of muscle, a portion of vertebral column, and the remainder of each dissected specimen (remaining sample) were examined (excluding heart, brain, liver, gonads, hemal arcs, and caudal vertebrae's). The gender and sexual maturity of each specimen was recorded, after being macroscopically determined. Next, tissues were dried at 60 °C (Universal Oven UFP 800, Memmert GmbH + Co. KG, Germany) for 72 h.

Metal determination

A set of 228 individual samples was chosen. The selection of the 228 samples was effectuated before analysis (Tables 1 and 2). A total of 228 samples (Tables 1 and 2) were tested for Cr, Cu, and Zn concentration. The remaining samples comprised the major amount of the samples. It was necessary to have a clearer idea about the whole fish that precludes accurate determination of human exposures. Cd was tested in 74 out of 228 samples because of its low concentrations, which covered a satisfactory range of the tissues (Tables 1 and 2).

Samples were wet digested in a microwave oven (Microwave 3000, Anton Paar GmbH, Austria) and stored at −4 °C until metal analysis was performed. The EPA 3052 protocol (standard procedure according to the US Environmental Protection Agency) was used for this procedure. Concerning digestion, 0.5 g of powered fish sample was digested using 3 ml of hydrofluoric acid (39.5 %) and 9 ml of nitric acid (65 %) in 16 medium-pressure vessels for 15 min. This method is appropriate for applications requiring a total decomposition for research purposes. After cooling, vessel contents were filled up to the desired volume (50 ml) using double-distilled water before storage. The results were expressed in a dry weight basis (in milligrams per kilogram dry weight) in order to avoid errors associated with moisture levels in tissues.

An atomic absorption spectrometer (PerkinElmer, AAnalyst 400 atomic absorption spectrometer, USA) was used to determine metal concentrations. Zn concentration was measured by using the flame (air-acetylene) technique (flame atomic absorption spectrometry; FAAS), while Cu, Cr, and Cd were measured by using the graphite furnace technique (graphite furnace atomic absorption spectrometry (GFAAS), HGA 900 equipped with an Autosampler 800). The detection limit for Cd was 0.04 μg/L. All samples were

Fig. 1 Sampling area in Pagasitikos Gulf, Greece
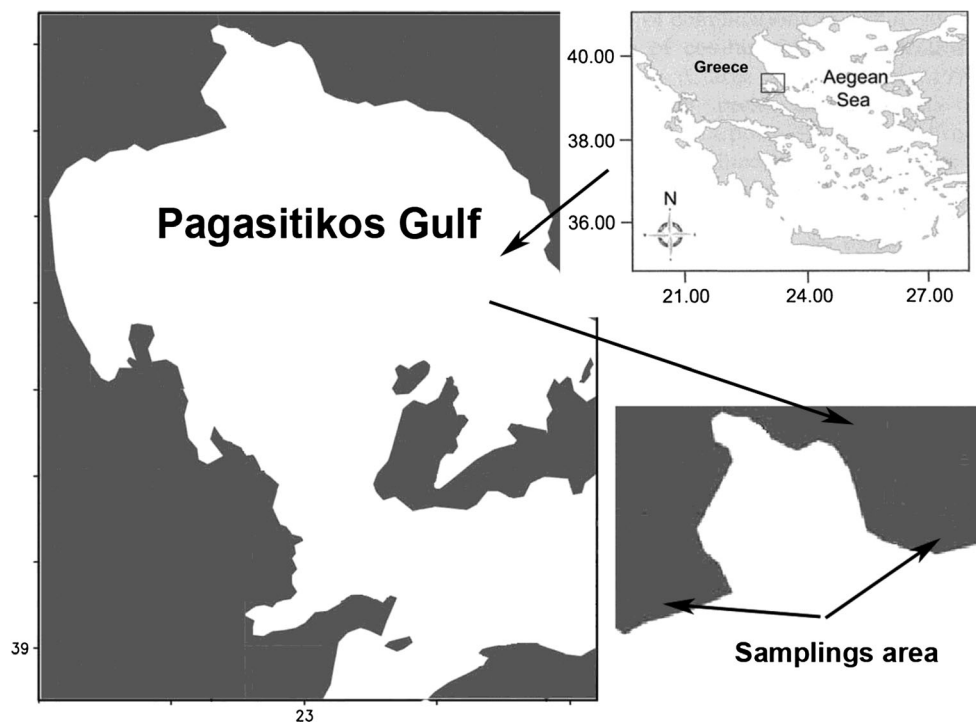
analyzed three times for metal detection using the FAAS and twice using the GFAAS.

The matrix modifiers for metal analysis was magnesium nitrate hexaydrate matrix modifier (Mg $(NO_3)_2$)·$6H_2O$ and palladium—magnesium nitrate hexaydrate matrix modifier (Pd + Mg $(NO_3)_2$·$6H_2O$). For a 5 µl matrix modifier addition, 1.75 g (Mg $(NO_3)_2$)·$6H_2O$ (10.5 %) and 3 g/L (0.3 %)

Pd+2 g/L (2.1 %) Mg $(NO_3)_2$·$6H_2O$ were used. Standards were prepared daily, as blanks and samples, using commercial materials (STD—AS WASTE WTR POLL 15 METAL), and they were used to check for matrix effects by running a standard additional method. A deuterium background correction was applied for determinations using the GFAAS.

Table 1 Information on the samplings of the selected specimens for metal determination (months, size classes, size classes per season)

	Mullus barbatus		*Pagellus erythrinus*
Remaining sample $n=85$	Specimens	Remaining sample $n=74$	Specimens
Class	I: 51.76 %, II: 43.53 %, III: 4.71 %	Class	I:37.84 %, II:48.65 %, III:13.51 %
Month (n)	Sep: 5, Oct: 7, Nov: 10, Dec: 13, Jan: 5, Feb: 3, Mar: 7, Apr: 8, May: 8, Jun: 5, Jul: 5, Aug: 6	Month (n)	Sep: 12, Oct: 5, Nov: 3, Dec: 6, Jan: 5, Feb: 3, Mar: 8, Apr: 8, May: 8, Jun: 5, Jul: 5, Aug: 6
Size classes per season	Autumn: small, 17.65 %; medium, 14.12 %; Winter: small, 9.41 %; medium, 9.41 %; large, 2.35 %; Spring: small, 17.65 %; medium, 9.41 %; large, 2.35 %; Summer: small, 7.06 %; medium, 10.59 %	Size classes per season	Autumn: small, 10.81 %; medium, 10.81 %; large, 5.41; Winter: small, 8.11 %; medium, 9.46 %; large, 1.35 %; Spring: small, 8.11 %; medium, 17.57 %; large, 6.76 %; Summer: small, 10.81 %; medium, 10.81 %
Muscle $n=21$	Specimens	Muscle $n=18$	Specimens
Class	I:47.62 %, II:52.38 %	Class	I: 22.22 %, II: 50.00 %, III: 27.78 %
Month (n)	Sep: 6, Oct: 6, Nov: 7, Dec: 2	Month (n)	Sep: 8, Oct: 7, Nov: 2, Dec: 1
Vertebral column $n=9$	Specimens	Vertebral column $n=11$	Specimens
Class	I:22.22 %, II:77.78 %	Class	I: 18.18 %, II: 63.64 %, III: 18.18 %
Month (n)	Sep: 5, Oct: 3, Dec: 1	Month (n)	Sep: 5, Oct: 3, Nov: 3
Gills $n=5$	Specimens	Gills $n=5$	Specimens
Class	I: 80.00 %, II: 20.00 %	Class	II: 20.00 %, III: 80.00 %
Month (n)	Sep: 4, Dec: 1	Month (n)	Sep: 2, Oct: 3

Table 2 Fish characteristics (length, weight, indexes, sex, maturity stage, moisture content) for *Mullus barbatus* and *Pagellus erythrinus* in Pagasitikos Gulf

	Mullus barbatus			*Pagellus erythrinus*	
Remaining sample *n*=85	Specimens		Remaining sample *n*=74	Specimens	
TL ± StDev	16.53±2.70 cm	Limits (12.20–27.60)	TL ± StDev	16.83±3.25 cm	Limits (10.90–25.00)
W ± StDev	55.87±32.76 g	Limits (17.60–243.40)	W ± StDev	70.80±43.97 g	Limits (18.59–221.70)
K ± StDev	1.99±0.75		K ± StDev	1.88±0.77	
GSI ± StDev	2.08±1.84		GSI ± StDev	0.80±1.01	
HSI ± StDev	2.00±1.68		HSI ± StDev	0.49±0.24	
Sex	Males: 15.3 %; Females: 31.8 %; Undetermined specimens: 52.9%		Sex	Males: 6.8 %: Females: 45.9 %; Undetermined specimens: 47.3 %	
Maturity stage	Immature, 8.24 %; resting stage, 9.41 %; developing stage, 10.59 %; maturing stage, 15.29 %; mature stage, 10.59 %; spent stage, 45.88 %		Maturity stage	Immature, 21.62 %; resting stage, 22.97 %; developing stage, 18.92 %; maturing stage, 2.70 %; mature stage, 5.41 %; spent stage, 28.38 %.	
Moisture content	29.7 %		Moisture content	30.8 %	
Muscle *n*=21	Specimens		Muscle *n*=18	Specimens	
TL ± StDev	15.94±1.45 cm	Limits (13.80–18.20)	TL ± StDev	17.22±2.78 cm	Limits (13.90–24.00)
W ± StDev	50.37±18.65 g	Limits (24.45–85.85)	W ± StDev	83.29±45.70 g	Limits (23.42–195.54)
K ± StDev	1.28±0.52		K ± StDev	2.35±1.45	
GSI ± StDev	0.41±0.31		GSI ± StDev	0.43±0.19	
HSI ± StDev	1.04±0.63		HSI ± StDev	0.48±0.19	
Sex	Males: 9.52 %; Females: 14.29 %; Undetermined specimens:76.19 %		Sex	Undetermined specimens: 27.78 %; Males: 5.56 %; Females: 66.67 %	
Maturity stage	Resting maturity stage: 19.05 %; developing stage: 4.76 %; spent stage: 76.19 %		Maturity stage	Resting maturity stage: 55.56 %; immature stage: 11.11 %; developing stage: 16.67 %; spent stage: 16.67 %	
Moisture content	23.1 %		Moisture content	22.3 %	
Vertebral column *n*=9	Specimens		Vertebral column *n*=11	Specimens	
TL ± StDev	16.46±1.70 cm	Limits (13.80–18.50)	TL ± StDev	18.15±3.44 cm	Limits (13.90–25.50)
W ± StDev	63.20±15.13 g	Limits (36.89–81.22)	W ± StDev	83.96±55.42 g	Limits (34.83–221.70)
K ± StDev	1.52±0.62		K ± StDev	1.85±0.54	
GSI ± StDev	0.43±0.35		GSI ± StDev	0.40±0.20	
HSI ± StDev	1.06±0.38		HSI ± StDev	0.44±0.18	
Sex	Undetermined specimens: 77.78 %; Females: 22.22 %		Sex	Undetermined specimens: 36.36 %; Females: 63.64 %	
Maturity stage	Immature: 11.11 %; resting stage: 22.22 %; spent stage: 66.67 %		Maturity stage	Resting stage: 45.45 %; developing stage: 18.18 %; spent stage: 36.63 %	
Moisture content	41 %		Moisture content	37.4 %	
Gills *n*=5	Specimens		Gills *n*=5	Specimens	
TL ± StDev	16.08±1.45 cm	Limits (14.80–18.50)	TL ± StDev	21.78±3.88 cm	Limits(16.90–25.50)
W ± StDev	49.26±22.20 g	Limits (24.45–81.22)	W ± StDev	152.36±55.30 g	Limits (98.56–221.70)
K ± StDev	1.18±0.41		K ± StDev	2.13±0.48	
GSI ± StDev			GSI ± StDev	0.44±0.14	
HSI ± StDev	0.87±0.43		HSI ± StDev	0.51±0.13	
Sex	Undetermined specimens: 100 %		Sex	Females: 100 %	
Maturity stage	Spent stage: 100 %		Maturity stage	Resting maturity stage: 40.00 %; developing stage: 60.00 %	
Moisture content	29.6 %		Moisture content	27.0 %	

The accuracy of measurements was examined by preparing and analyzing a standard solution with known concentration (2 ppb), which was then run as sample. The accuracy of the analytical procedure was satisfactory

for all metals (85.8±3.6 %). An additional procedure, which involved spike blanks, was used to certify the method's accuracy (recovery, 95.4±4.6 %).

Statistical analysis

All statistical analyses were performed using the SPSS 17.0 (IBM, USA) software. The Kolmogorov–Smirnov and Shapiro–Wilk tests were utilized to analyze the normality of the data distribution.

One-way analysis of variance (ANOVA) was used to test for significant differences of the metal concentrations among each species, and tissues, seasons, gender, classes, and level of maturity or testing differences between species. Tukey's t test was applied in all cases to check for possible differences between groups (Zar 1996).

When the distribution fitting was abnormal, nonparametric statistics was used (Kruskal–Wallis). Furthermore, Independent samples t test (or Mann–Whitney test in nonparametric cases) was used to identify all the possible differences between examined groups, when Tukey's HSD post hoc test could not be performed.

A paired samples t test was used to compare this study's findings with other related studies (e.g., Panayotidis and Florou 1994; Catsiki and Strogyloudi 1999). Values less than 0.05 (5 %) were considered to be statistically significant ($P<0.05$). Coefficient of variation (CV) was performed in order to measure variables' dispersion.

Risk assessment for human consumption

The maximum safe consumption (MSC) was calculated depending on mean concentrations of Cr, Cu, Zn, and Cd in the muscle of both species. Maximum safe fish consumption was evaluated on the provisional maximum tolerable daily intake (PMTDI) or the provisional tolerable weekly intake (PTWI) given by the Joint Expert Committee of Food Additives (JEFCA; http://www.inchem.org/pages/jecfa.html). The maximum provisional tolerable intake for Cd is 0.007 mg/kg body weight (bw) per week, 3.5 mg/kg bw/week, 7 mg/kg bw/week, and 1.4 mg/kg bw/week (JEFKA) for Cu, Zn, and Cr, respectively. Metal concentrations from other food sources or drinking water, except metal concentrations in fish meals, were not calculated in the results (Metian et al. 2013).

Male and female human average weight (80 and 60 kg, respectively) was used to calculate the PTWI in order to obtain the "maximum safe consumption" for muscle samples in both species. The following equation was used (Metian et al. 2013): $MSC_A = (W_{ind}*JL_A)/X_A$ to calculate MSC_A. MSC_A is the maximum safe consumption by the JEFCA (in milligrams per kilogram wet weight). X_A is the mean concentration of the tested element. W_{ind} is human's body weight, and JL_A represents PMTDI or PTWI. MSC_A results give the maximum

fish mass (in grams) allowed per day or per week for human consumption.

Results

Significant differences in Cu and Zn concentration (Fig. 2) were observed in red mullet's tissues (Kruskal–Wallis, $df=4$; $P<0.05$). The same was noticed for Zn and Cd concentrations (Fig. 2) in common pandora's tissues (Kruskal–Wallis test, $df=4$; $P<0.05$). Furthermore, the gender is not statistically significant in metal bioconcentration between red mullet's and common pandora's individual organisms samples, except Cd concentrations (Kruskal–Wallis, $df=2$; $P<0.05$). Additionally, Cu concentrations have significant differences between the two species (Kruskal–Wallis, $df=1$; $P<0.05$), and the same was observed in Cu, Zn, and Cd concentrations (Fig. 2) among different species tissues (Kruskal–Wallis, $df=4$; $P<0.05$).

The factor seasonality is statistically significant in Cu and Zn concentrations for red mullet's remaining samples (Kruskal–Wallis, $df=3$; $P<0.05$), while it is significant for all metal concentrations for $P.$ $erythrinus$ remaining samples (Kruskal–Wallis; Cr, Cu, Zn, $df=3$; Cd, $df=1$; $P<0.05$). In particular, the largest values in Cu concentrations were noticed in autumn and spring, for red mullet's remaining samples, while red pandora's remaining samples had the largest Cu values in spring (Fig. 5). Individuals of $M.$ $barbatus$ were observed to have the maximum Cu concentrations in autumn, with almost stable values till spring, and after this, a drop-off value continued till summer (Fig. 5). On the contrary, in $P.$ $erythrinus$, individuals observed an anodic trail till spring and a fall after this (Fig. 5).

For both species, in Zn concentrations, maximum values were observed in autumn (Fig. 5), while red mullet had almost the same values as autumn in spring. Both species followed the same value trend in Zn concentrations as autumn > winter and spring > summer. Cu and Zn concentrations were found to have statistically significant differences among summer and all the other seasons (Fig. 5) for both species (Mann–Whitney test, $P<0.05$), and the same among months of samplings (Fig. 4) of $M.$ $barbatus$ and $P.$ $erythrinus$ individuals (Mann–Whitney test, $P<0.05$).

In $M.$ $barbatus$ remaining samples, in Cr concentrations, significant differences were found between autumn and winter (Mann–Whitney, $P<0.05$), while in winter, we recorded the maximum values (Table 3; Figs. 3, 4, and 5). However, in winter, the minimum Cr values were observed in $P.$ $erythrinus$ remaining samples (Table 3; Figs. 3, 4, and 5). Maximum Cr values in $P.$ $erythrinus$ remaining samples were observed in spring (Fig. 5), and the spring factor was significantly different from all the other seasons (Mann–Whitney test, $P<0.05$). Both species showed a fall in their Cr values in summer. In Cd concentrations, largest values were observed in autumn and the smallest in summer for $P.$ $erythrinus$ (Table 3; Figs. 3, 4, and 5). Significant differences were found in all metal concentrations in $P.$ $erythrinus$ remaining
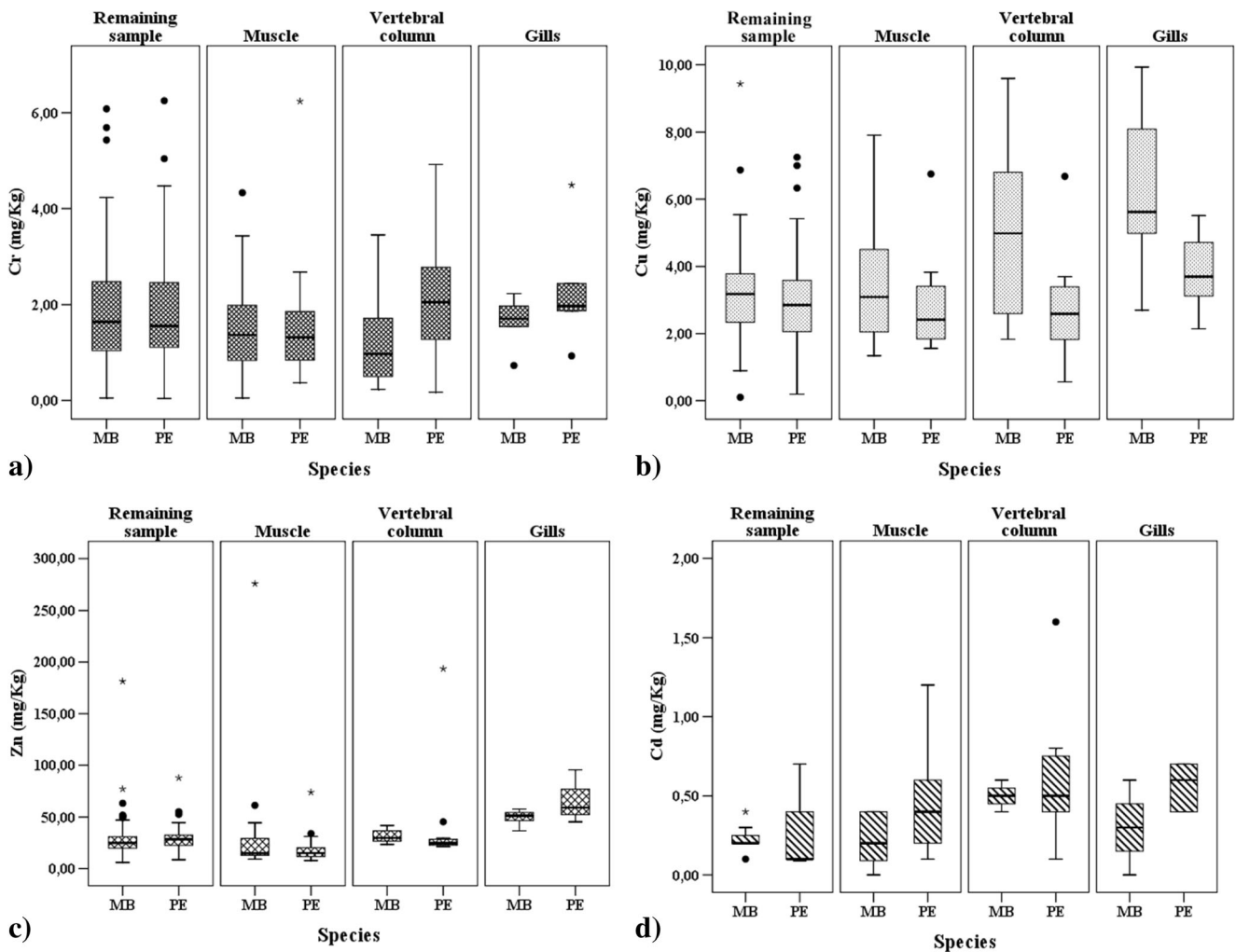
MB: *Mullus barbatus*
PE: *Pagellus erythrinus*

Fig. 2 Box plot diagrams (Median, SE) of metal concentrations in the studied tissues of *Mullus barbatus* (MB) and *Pagellus erythrinus* (PE)

samples among months of samplings (Fig. 4). Differences that were found among seasons (or among months) in tissues may reflect differences in polluting sources, except each fish species characteristics (Dural et al. 2010).

Furthermore, size class was not a statistically significant parameter for metal concentrations in *M. barbatus* (Kruskal–Wallis; Cr, Cu, Zn, $df=2$; Cd, $df=1$; $P>0.05$) and *P. erythrinus* remaining samples (Kruskal–Wallis, $df=2$; $P>0.05$), while individuals showed significant differences (Table 3) in Zn concentrations (Kruskal–Wallis, $df=2$; $P<0.05$) in *P. erythrinus* andbetween species. Differences were found only between small and medium sized individuals in both species, in Zn concentrations, and between small and large individuals, in *P. erythrinus*, in Cr concentrations(Mann–Whitney, $P<0.05$). Between species (Table 3, Fig. 6), differences were observed between small- and medium-sized individuals (Table 1) in Zn concentrations (Mann–Whitney, $P<0.05$).

Moreover, in *M. barbatus*, small individuals had maximum Cr values in summer, while medium-sized in spring and large-sized in winter (Fig. 6). Alongside, small, medium, and large specimens of red pandora had the maximum Cr values in spring. In red mullet's Cu concentrations, the maximum values were observed in autumn, in small-sized specimens, and the same found in red pandora (Fig. 6). On the contrary, the highest Cu values in *M. barbatus* were noticed in winter, in medium-sized, and in spring in large-sized specimens. The same happened for large specimens of red pandora because spring values were the highest, as well as in medium-sized individuals. Similar values were observed in autumn and winter in red pandora's small-sized and medium-sized specimens (Fig. 6).

In *M. barbatus*, in Zn concentrations, the small- and medium-sized individuals gained the biggest values in autumn, while the large specimens were observed to have the highest values in winter, although autumn and winter values
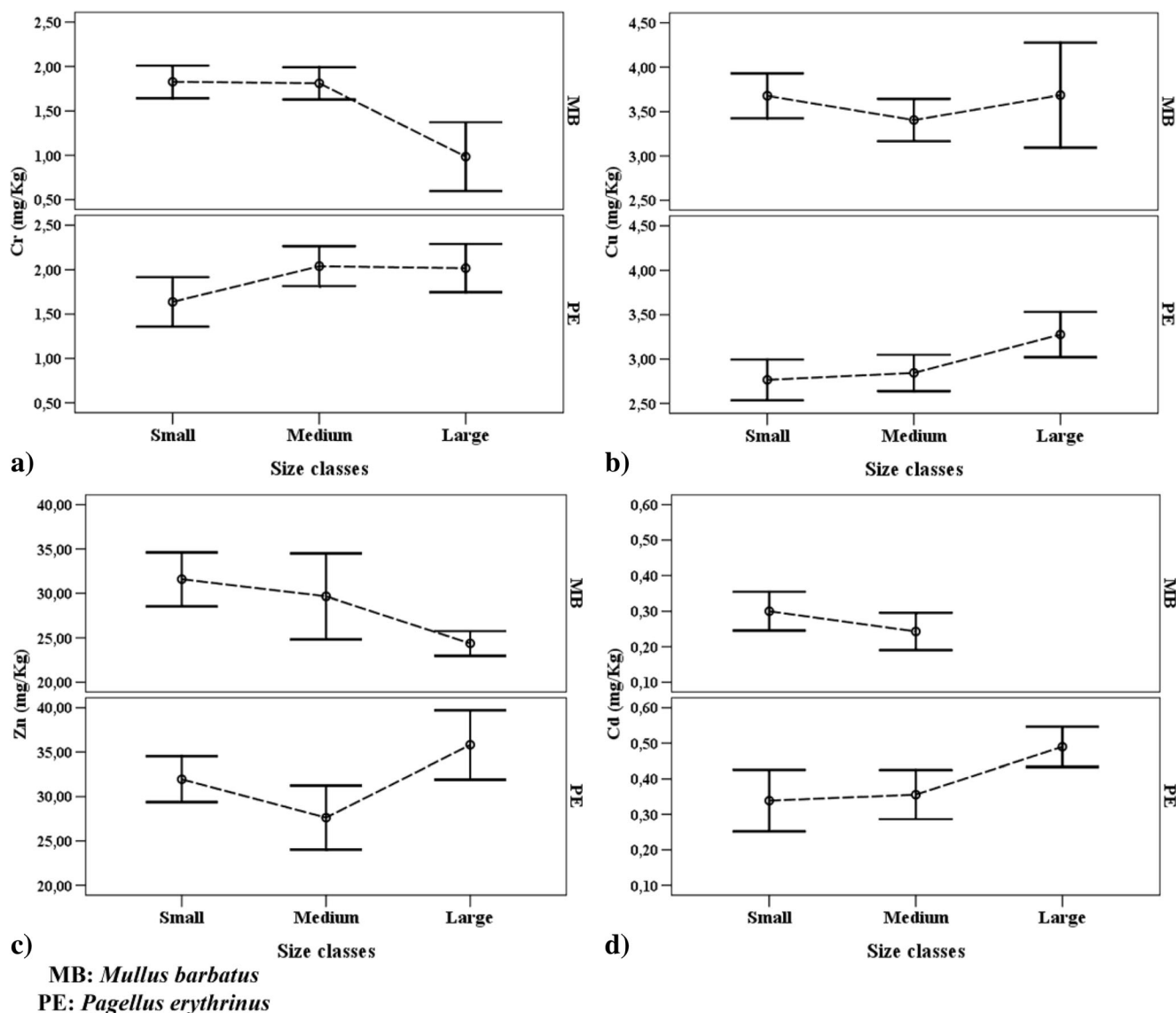
Fig. 3 Metal concentrations in "remaining samples" of *Mullus barbatus* (MB) and *Pagellus erythrinus* (PE) according to size classes (Mean, SE±1)

had similar concentrations (Fig. 6). Autumn picked up the largest Zn concentrations, in both species, in small-sized specimens. In medium-sized specimens of red mullet, Zn concentrations were the highest in autumn, though in red pandora the highest were in winter. Red pandora's large specimens showed the largest values in spring while the highest values in red mullet's large specimens were in winter, although winter obtained similar values to spring in red mullet's large specimens (Fig. 6). In autumn, bigger Cd values were found in both species, in small-sized individuals. Seasonality was observed in the same size classes (Kruskal–Wallis test, $df=3$; $P>0.05$).

Sex does not play an important role in metal concentrations in the remaining samples of *M. barbatus* (Kruskal–Wallis; Cr, Cu, Zn, $df=2$; Cd, $df=1$; $P>0.05$) or *P. erythrinus* (Kruskal–Wallis, $df=2$; $P>0.05$). The same was observed for the

maturity stage (Kruskal–Wallis; Cr, Cu, Zn, $df=5$; Cd, $df=1$; $P>0.05$), although statistically significant differences in Zn concentrations, in red mullet's remaining samples, were found between maturing and mature stages (Fig. 7) and between mature and spent stages (Mann–Whitney test, $P<0.05$).

Additionally, differences in Cd concentrations were observed only between red pandora's undetermined specimens and the female specimens (Mann–Whitney, $P<0.05$), while maturity stage (Fig. 7) of the specimens was important for Zn and Cd concentrations (Kruskal–Wallis, Zn, $df=5$; Cd, $df=4$; $P<0.05$). Differences were found in Zn concentrations between immature and mature stages, resting and mature stages, developing and mature stages (Fig. 7), as well as between mature and spent stages (Mann–Whitney test, $P<0.05$) in red pandora's remaining samples. In Cd concentrations, differences were noticed between immature and resting stages

Table 3 Descriptive statistics of metal concentrations (mg/kg dry weight) in two species

	Mullus barbatus				Pagellus erythrinus			
Remaining sample	n=85				n=74			
	Cr	Cu	Zn	Cd (9)*	Cr	Cu	Zn	Cd (29)*
Males	2.51	2.95	29.73		2.24	2.60	25.43	0.13
Females	1.94	3.22	24.03	0.25	2.08	3.11	28.29	0.37
Undetermined specimens	1.72	3.29	28.02	0.22	1.78	2.73	30.35	0.18
Overall Mean ± StDev	1.92±1.32	3.21±1.36	27.02±12.76	0.23±0.10	1.97±1.32	2.90±1.38	29.07±11.58	0.25±0.22
CV%	69	43	11	41	67	48	40	88
ND**	20	1			24	1		
Muscle	n=21				n=18			
	Cr	Cu	Zn	Cd (7)*	Cr	Cu	Zn	Cd (10)*
Overall Mean ± StDev	1.61±1.11	3.57±2.00	24.63±20.70	0.20±0.16	1.58±1.31	2.78±1.24	20.27±15.45	0.40±0.21
CV%	69	56	84	80	83	45	76	53
ND**	2	1						1
Vertebral column	n=9				n=11			
	Cr	Cu	Zn	Cd (4)*	Cr	Cu	Zn	Cd (7)*
Overall Mean ± StDev	1.26±1.08	5.14±2.82	32.07±6.82	0.50±0.08	2.16±1.44	2.73±1.66	29.88±13.34	0.64±0.48
CV%	86	55	21	16	67	61	45	75
ND**					3			
Gills	n=5				n=5			
	Cr	Cu	Zn	Cd (3)*	Cr	Cu	Zn	Cd (5)*
Overall Mean ± StDev	1.64±0.57	6.26±2.81	49.35±8.23	0.30±0.30	2.34±1.32	3.84±1.32	65.87±20.28	0.56±0.15
CV%	35	45	17	100	56	34	31	27
ND**								

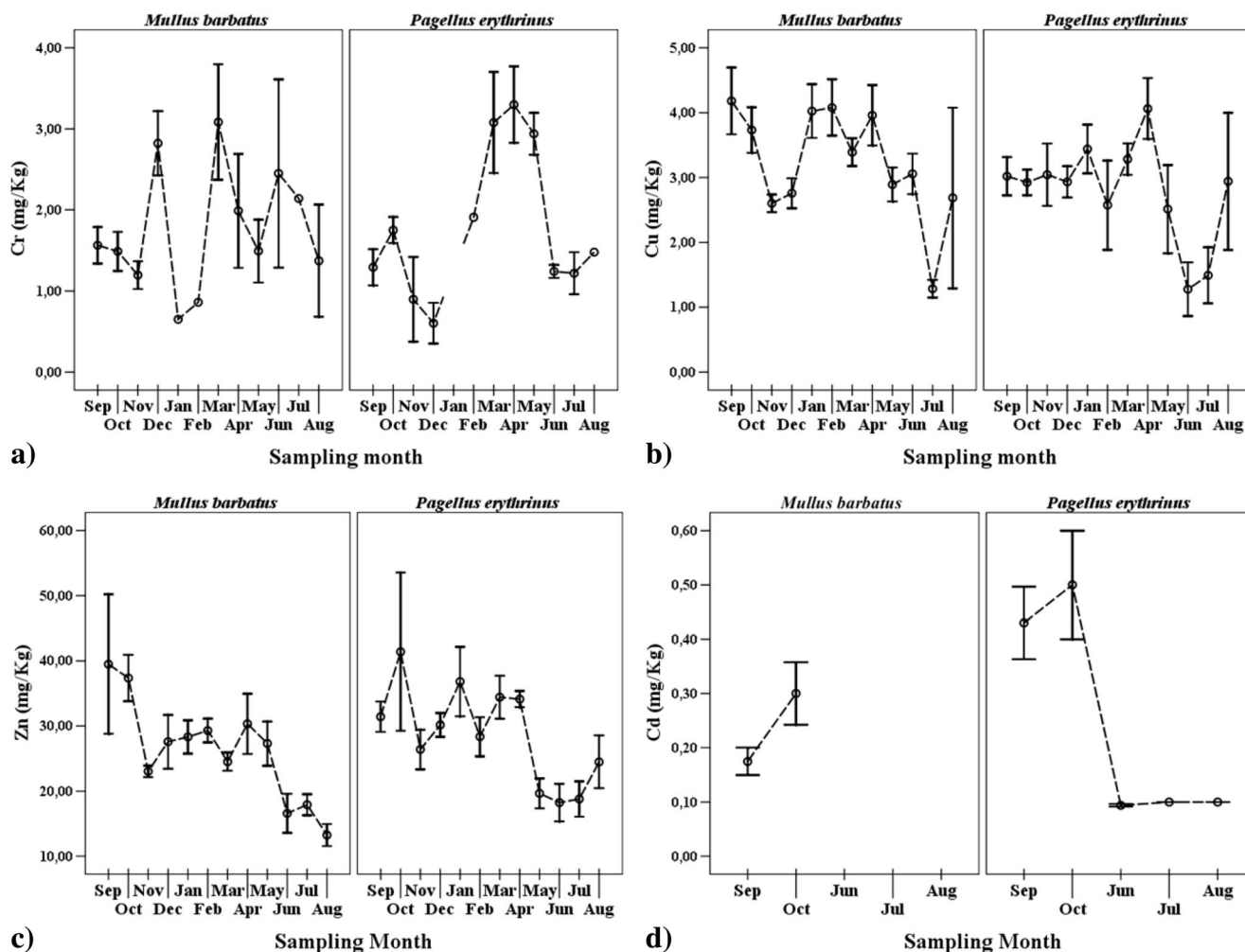*Number of checked specimens for Cd concentrations, **ND *Not detected*

Fig. 4 Metal concentrations in "remaining samples" of *Mullus barbatus* and *Pagellus erythrinus* according to month of the sampling (Mean, SE±1)

(Fig. 7), and between immature and spent stages (Mann–Whitney test, $P<0.05$). In Cr concentration, differences emerged only between resting and developing stages (Mann–Whitney test, $P<0.05$). In both species, the most metal concentrations showed an increase till maturing stage and a drop-fall in mature stage (Fig. 7).

The class parameter was not statistically significant between size classes (Independent Samples t tests; Mann–Whitney tests; $P>0.05$) in red mullet's (ANOVA; Cr, $df=18$; $F=3.157$; Cu, $df=20$; $F=1.579$; Cd, $df=8$; $F=0.003$; Kruskal–Wallis, Zn; $P>0.05$) or red pandora's muscle samples (ANOVA; Cu, $df=17$; $F=2.436$; Kruskal–Wallis; Cr, Zn, Cd; $P>0.05$). There were also statistically significant differences between September and November, and between October and November, in Cu and Zn concentrations (Fig. 2), of red mullet muscle samples, as well as between October and November , in Zn concentrations, in red pandora's muscle samples(Mann–Whitney test, $P<0.05$). Significant differences in sex were observed only between undetermined and male specimens, in red mullet's muscle samples, and between resting and spent stages (Fig. 7), in red pandora's

muscle samples, in Zn concentrations (Mann–Whitney test, $P<0.05$). No statistically significant differences were observed within species in column vertebral and gill samples related to class size, gender, or maturity stage ($P>0.05$).

Both species' tissues appear to have high CV (%) values in most cases, and this means that in the majority of cases the data are more widespread; in more than 50 % of the samples, meaning there was very high variability between samples, which is mainly due to handling of the two species, the sampling sites or the collection time (Catsiki and Strogyloudi 1999). CV values represent a more individual approach for each specimen rather than a species-oriented approach.

Risk assessment for human consumers' results

The MSC was estimated based on the mean Cr, Cu, Zn, and Cd concentrations in red mullet's and common pandora's muscle tissues. MSC was calculated in two ways as follows: on a daily and a weekly basis. The recommended intake by JECFA (WHO 2003) for each contaminant was taken into account.
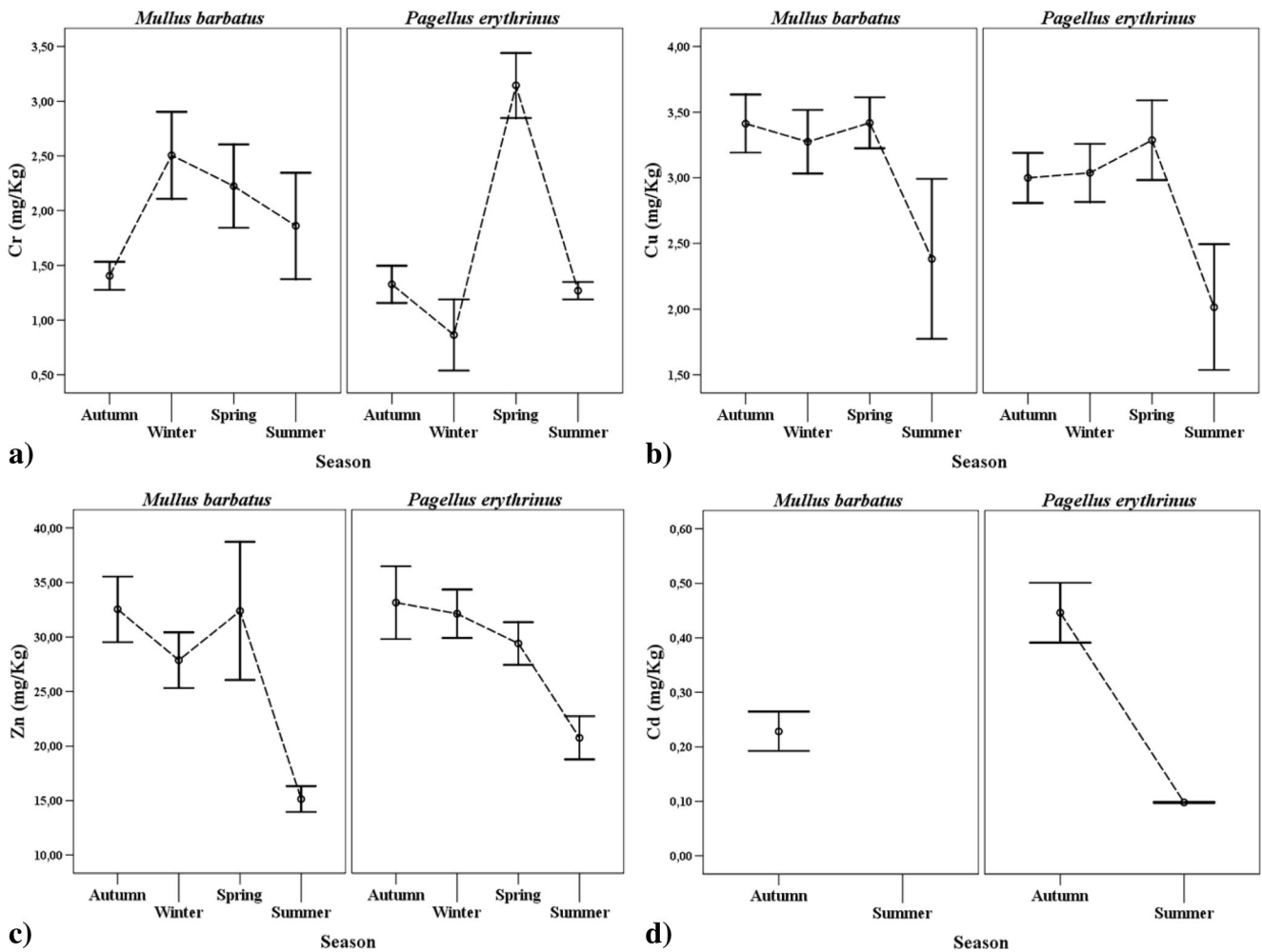
Fig. 5 Metal concentrations in "remaining samples" of *Mullus barbatus* and *Pagellus erythrinus* according to season (Mean, SE±1)

The "maximum safe daily consumption" (MSDC) for human consumption (fish muscle) of *M. barbatus* (edible part) was above 150 g in women (MSDC, in grams wet weight daily), and exceeded 200 g in men (Cr 12,515 g, Cu 10,496, Zn 1,771 g, and Cd 155 g in women; Cr 16,686 g, Cu 13,994, Zn 2,362 g, and Cd 207 g in men). Furthermore, the MSDC for *P. erythrinus*' edible parts exceeds 160 g in women and 220 g in men (Cr 9,799 g, Cu 13,900, Zn 3,810 g, and Cd 166 g in women; Cr 13,065 g, Cu 18,533, Zn 5,079 g, and Cd 221 g in men).

The maximum amount of *M. barbatus* muscle that can be consumed by a 60-kg woman to reach the PTWI for Cd is 1,087 g over a week; while in an 80-kg man is 1,450 g. Furthermore, the largest amount of *P erythrinus* muscle consumed by a 60-kg woman to reach the PTWI for Cd is 1,158 g over a week; while in an 80-kg man, this is 1,544 g.

Discussion

This paper investigated whether four different heavy metals show similar temporal patterns of accumulation in various tissues of two sympatric fish with different feeding modes. Feeding mode is related to the uptake and fate of the metals in the organisms. We also investigated whether these patterns were affected by season, age, or size class in each species in order to detect how seasonality affects metal accumulation or which age/size class is more prone to heavy metal accumulation.

Non-statistically significant differences of heavy metals concentration were observed in the majority of the cases in small- or medium-sized fish in both species (Fig. 3). Small differences were noticed in different development stages during fish growth in both fish species (Fig. 7). In some tissues (e.g., remaining sample), a small metal concentration decrease (e.g., Cu, Zn) in fish length was noticed during development or the concentration was just stable. The decrease of metal concentrations in fish growth was in contrast with fish size that depends on higher metabolic rates in juveniles (Gaspic et al. 2002).

Metal bioaccumulation data showed Cr, Cu, and Zn levels in flesh similar to other related studies (Catsiki and Strogyloudi 1999). Catsiki and Strogyloudi (1999) reported Cr and Cu levels (e.g., Cr 0.06–11.8 mg/kg d.w.;
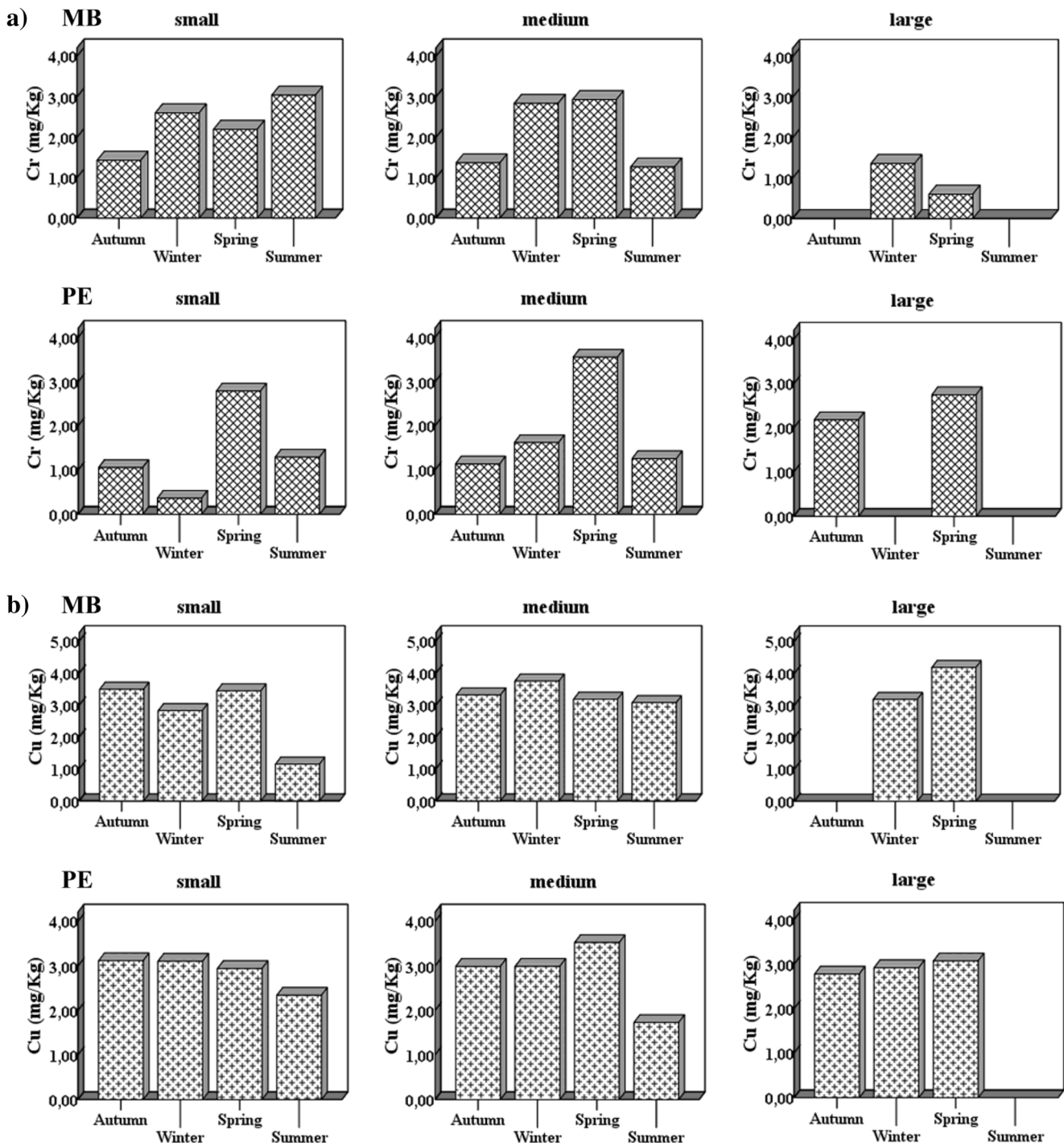
Fig. 6 Metal concentrations in "remaining samples" of *Mullus barbatus* and *Pagellus erythrinus* according to season and size classes (Mean)

Cu 0.06–15.5 mg/kg d.w.; Zn 0.8–76.0 mg/kg d.w.) in red mullet's muscle in agreement with our study's findings (paired samples *t* test, $df=2$; $P>0.05$), as well as Cicek et al.'s (2008) study for common pandora's muscle (e.g., Cu 0.69±1.71 mg/kg of wet weight). In this study, Cd was present in low concentrations in fish muscle (0.00–0.40 mg/kg d.w. in red mullet and 0.10–0.70 mg/kg

d.w. in common pandora). In most cases, Cd concentration was lower than in other related studies. Panayotidis and Florou (1994) reported 0.4–1.0 mg/kg of Cd in *M. barbatus* flesh (paired samples *t* test, $df=2$; $P<0.05$), while Turan et al. (2009) results showed Cd level in muscle ranging between 0.311 and 0.697 µg/g d.w. (paired samples *t* test, $df=2$; $P<0.05$).
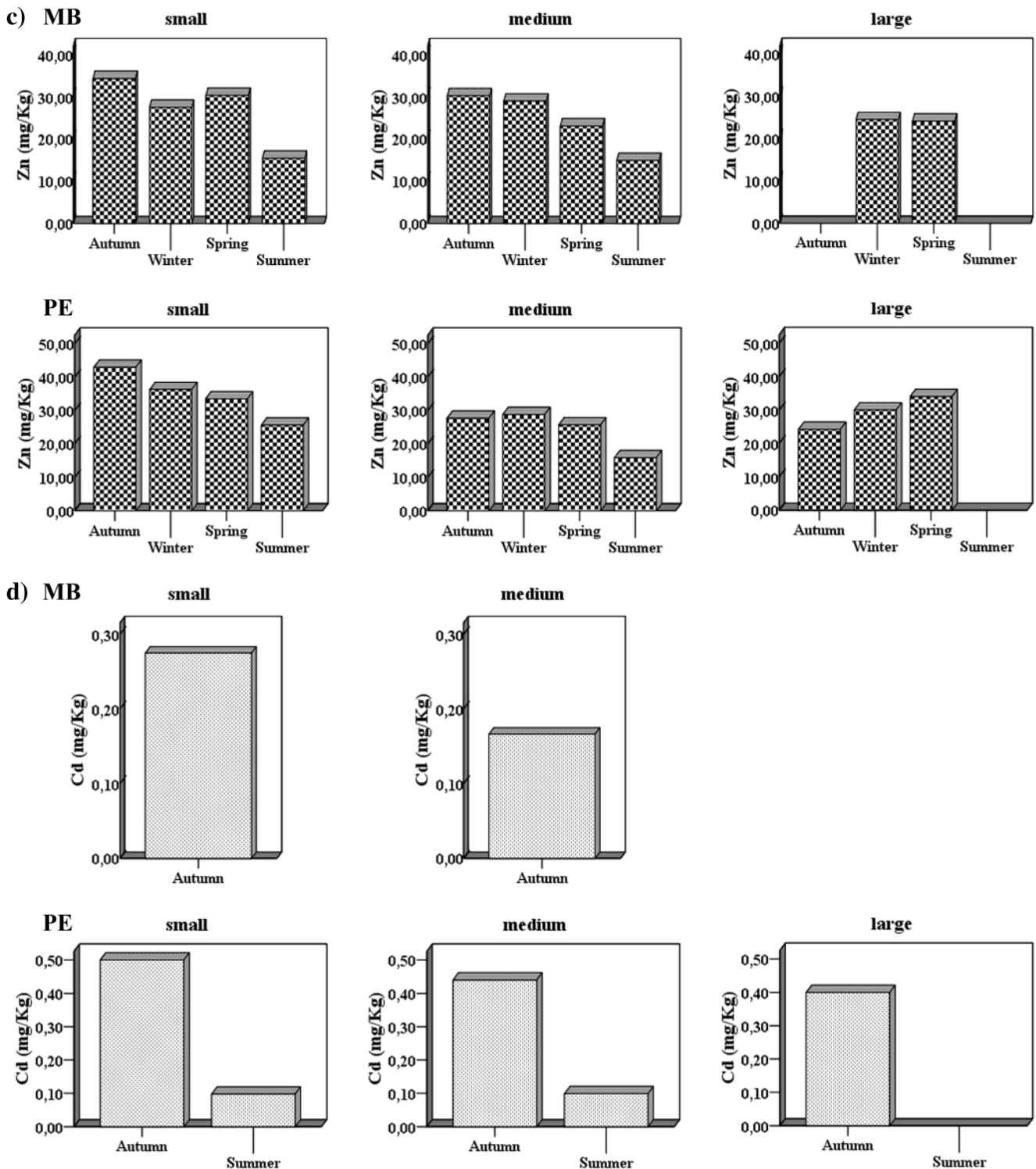
Fig. 6 continued.

Variables for gills showed some differences only in Cr concentrations (paired samples *t* test, $P>0.05$) than in other parameters' results (Vlachonis 2002). Zn concentrations results in *M. barbatus* gill samples are in disagreement (paired samples *t* test, $df=2$; $P<0.05$) with Zyadah and Chouikhi (1999), but Cu and Cd concentrations agree. Cu and Zn concentrations in

P. erythrinus gill samples coincide with Cicek's et al. (2008) winter results for 2004. Vertebral column's concentrations in *P. erythrinus* tissues are higher than Khalifa et al.'s (2010) results in bones. *M. barbatus* column vertebrae's samples are in agreement with other related studies in other species, except Cd concentrations (Beltcheva et al. 2011).
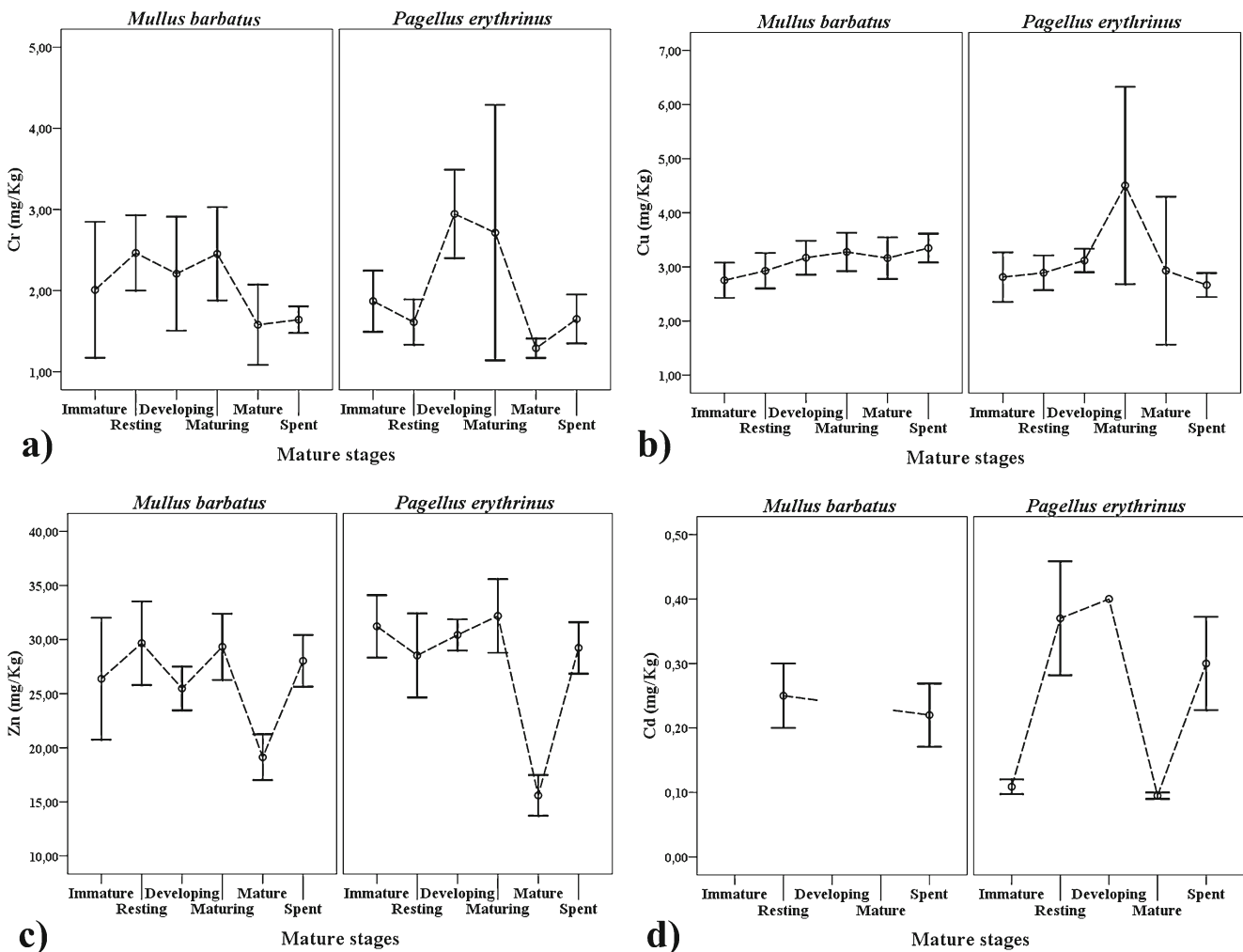
Fig. 7 Metal concentrations in "remaining samples" of *Mullus barbatus* and *Pagellus erythrinus* according to maturity stage (Mean, SE±1)

The data demonstrates that sampling was statistically significant in metal's distribution in different tissues. This occurs because of the differences between species growth cycles or reproductive cycles and changes in water temperature among seasons' (Dural et al. 2010). Season variability is also an important factor, because it may influence the metal accumulation rate in fish tissues (Phillips 1980) High condition factor (K), gonadosomatic index (GSI), and hepatosomatic index (HSI) values showed that fish condition was good; gonads were larger than the other specimens' or each one had higher reproductive activity. This may also represent nutritional status; high HSI values imply an increased liver activity related to its' exposure to organic pollutants (Martinez-Gomez et al. 2012).

The maximum provisionally tolerable weekly intake for Cd was 0.5 mg/week (FAO 1983). The maximum permissible daily intake for Cu was 30 mg/kg, for Zn was 100 mg/kg, and for Cr was 50 mg/kg (WHO 1989). In almost all of our samples, the data demonstrates that there were low Cr, Cu, Zn, and Cd concentrations. This means that the fish is safe

food for human intake. Heavy metal concentrations in the edible parts of *M. barbatus* and *P. erythrinus* did not show a risk for human consumption.

Conclusion

Overall, this study provides information for metal concentrations in two commercial fish species and the differences that each one represents in their tissues' metal accumulation. This paper showed that seasonality is an important factor for both species because it can affect the accumulation of metals in their organisms. This is because of the ecological and biological characteristics of the species. The body size of the organisms does not play an important role in metal concentrations, but significant differences in metal concentrations between size classes observed. Metallic levels and tissues have an important correlation. Gender is not statistically significant for both species, but differences were observed among sexes and maturity stages of the same species.

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