Metal bioaccumulation in representative organisms from different trophic levels of the Caspian Sea

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Abstract

The bioaccumulations of metals Cu, Cd, Ni, Cr, Co, Mn, Zn and Fe were measured in bivalves, *Cerastoderma glucaum*, and four species of fishes including *Alburnus chalcoides*, *Liza aurata*, *Rutilus frisii* and *Sander lucioperca* from various trophic levels of the Caspian food web. The concentrations of Cd, Cr, Co and Ni in most samples of fish were below the detection limits; while the concentrations were detected in most samples of bivalve *C. glucaum*. The stable nitrogen isotope ratios varied among the samples from *C. glucaum* ($\delta^{15}N$=3.5 ‰) to *S. lucioperca* ($\delta^{15}N$=13.1‰). Among the four fish species, while the highest concentrations of Mn, Ni and Fe were observed in *L. aurata*, the lowest concentrations of Mn and Fe were observed in *S. lucioperca*. These species also had the lowest and highest trophic levels with an average of 3.3 and 4.2, respectively. No accumulation of metals with increase in body size was observed in muscles of species from different trophic levels. The comparison of metal concentrations with the health guidelines for human consumption showed that those intakes were lower than the legislated limits. While there was a strong relationship between trophic levels and body size of *A. chalcoides* and *R. frisii*, no significant slopes were observed between the total lengths (TLs) and the Ln concentrations of metals. It is necessary to determine metal concentrations in food resources of fish species, particularly in *R. frisii* that has significantly different $\delta^{15}N$ in relation to body size.

Keywords: Metal pollution, Bioaccumulation, Stable isotope, Caspian Sea

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Introduction

The Caspian Sea, the largest land-enclosed drainage area in the world, supports substantial fisheries, while it has been endangered by massive loads of contaminants. The various environmental pollutants produced by natural and anthropogenic sources are discharged from coastal catchment (Glantz, 2000; UNEP/UNDP, 2011). The hazardous effects of heavy metals on human health are reviewed by international organizations such as the WHO (Järup, 2003).

Caspian fishes, an important source of protein, are consumed by millions of people living around the Caspian Sea; therefore monitoring their metal pollution is essential (Fallah *et al.*, 2011).

Bioaccumulation is defined as an increase in the concentration of pollutants by an increase in age, which sometimes is reflected in size of the organism (Fernandes *et al.*, 2007). Metal bioaccumulation is influenced by various environmental and biological factors particularly the feeding source (Agusa *et al.*, 2004; Wang and Rainbow, 2008). Although referring to scientific literature, very few data have been published on metal accumulation related to size and age (Anan *et al.*, 2005; Pourang *et al.*, 2005; Foroughi Fard *et al.*, 2008; Pazooki *et al.*, 2009; Hoseini and Tahami, 2012). There have been some research (Sadeghirad, 2002; Agusa *et al.*, 2004; Sadeghirad, 2007; Shahryari *et al.*, 2010; Eslami *et al.*, 2011; Fallah *et al.*, 2011; Monsefrad *et al.*, 2012) presenting metals concentrations in the Caspian fishes. While nearly all of these reports were focused on the Acipenseridae species (particularly *Acipenser persicus*) and *Rutilus frisii*, other commercial fishes were less concerned. Furthermore, no research has been done according to trophic levels of Caspian fishes.

Four species, *A. chalcoides*, *Liza aurata*, *R. frisii* and *Sander lucioperca*, have a high commercial value and good market demands in the southern Caspian regions. These species are traditionally known as zooplankton feeders, detritivrous, benthivorous and piscivorous, respectively (Ghadirnejad, 1996; Zarinkamar, 1996; Abbasi *et al.*, 2004; Abdolmaleki and Ghaninezhad, 2007; Neamatparast, 2007).

Stable isotope analysis has been widely used, as a powerful tool, for source identification of changes in aquatic and estuarine ecosystems, and also for assessing pollutant flux in marine ecosystems (Broman *et al.*, 1992; Cabana and Rasmussen, 1996; Vander Zanden and Rasmussen, 1996; Lake *et al.*, 2001; Jardine *et al.*, 2006).

However the metal concentrations in muscle tissues are reported to be usually lower than in other organs (Pourang *et al.*, 2005; Kojadinovic *et al.*, 2007; Mashroofeh *et al.*, 2013), the turnover rate of $\delta^{15}N$ in muscle protein is much longer and represents the integrated diet and contamination of fishes for a long time (Cabana and Rasmussen, 1996; Vander Zanden and Rasmussen, 1996).

Typically the $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios of a consumer are slightly higher

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than those in its diet. On average, $\delta^{13}C$ and $\delta^{15}N$ values of consumers are expected to increase by around 1‰ and 3.4‰ compared to those of their food, respectively (Minagawa and Wada, 1984). The nitrogen isotope compositions are particularly well-suited for examining trophic dynamics and provide an estimate of their trophic position (Minagawa and Wada, 1984; Post, 2002) while carbon isotope elucidates the origin and pathways of organic matter in food webs (Le Loc'h *et al.*, 2008).

In this study, we determined metal concentrations in *A. chalcoides, L. aurata, R. frisii* and *S. lucioperca*. Furthermore, we studied the metal bioaccumulation based on the trophic levels in the Caspian food web.

Material and methods

Sample collection

The collections included Pike-perch (*S. lucioperca*) (n=16), Kutum (*R. frisii*) (n=25), Danube bleak (*A. chalcoides*) (n=20) and Golden grey mullet (*L. aurata*) (n=16). These species were freshly collected from Anzali region (~37 28 N 49 33 E) during winter-spring of 2013. Two fishing methods; beach seine and gill net (Gerami and Dastbaz, 2013) were used for sampling. Furthermore about 360 individuals of the benthic invertebrates bivalvia *C. glucaum* were freshly collected along the beach down to 20 meter depths by hand and by using a bottom sampler (Van-Veen grab: 400 cm²), respectively. The total length of each sample was measured by Vernier calipers; and then they were weighed using a balance at the nearest 0.01g precision.

The dorsal muscles of fish samples were individually dissected using a high quality stainless steel knife; rinsed with distilled water and dried at 60 °C for 48 h. The dried samples were ground and homogenized with a pestle and mortar, stored in distilled water washed in plastic bags and kept at -20°C before the laboratory experiments. Gut organs and shells of mollusks were carefully removed under stereomicroscope and their soft tissues were pooled (20-30 individuals as one sample). The samples were oven dried at 60 °C for 24 h. The preparation of samples followed the ROPME instructions (1989).

Chemical analyses

For metal (Cu, Cd, Ni, Cr, Co, Mn, Zn and Fe) analyses, a mass of around 0.5 g of each sample was digested in4 ml high purity nitric acid (Merck, 65%) and 1 mL perchloric acid (Merck, 70%) at room temperature. Digestion continued on a sand bath with a maximum temperature of 115 °C, for about 4 h and until the solutions were cleared. After cooling, the solutions were filtered through Whatman filter membranes and were diluted up to 10 ml in a volumetric flask with deionized distilled water. The digestion procedure followed the ROPME instructions (1989) and Pourang (1995, 1996).

A HP 4500 ICP-MS, equipped with Asx-520 Autosampler (England) was used for the determination of metal concentrations in the samples. In order to control the quality of the measurement for each 25 samples, 2 blanks and 2 spiked samples were used. Instrumental detection limits for most metals was 0.02 µg/g, except for Cd (0.01 µg/g) and Fe (0.1 µg/g). The recoveries for Cd, Cu, Co, Cr, Ni, Mn, Zn and Fe were 84%, 107%, 79%, 94%, 101%, 86%, 72% and 72%, respectively.

Stable isotope analysis and trophic level calculation

The stable isotope signatures were used to calculate the trophic levels. Around 1 mg of homogenized powder of each sample was weighed in tin capsules. No further steps were taken for the elimination of carbonates and lipids because this helps us to avoid dispersion of $\delta^{13}C$ and $\delta^{15}N$.

All stable isotope measurements were performed at the University of California, Davis, USA. Isotope values were expressed in standard d-notation relative to the international V-PDB (Vienna PeeDee Belemnite) for carbon and atmospheric N_2 for nitrogen, using the standard equation;

$\delta^{13}C$ or $\delta^{15}N = [(R_{Sample} / R_{Standard}) - 1] \times 1000$

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

The trophic level (TL) was calculated using the following equation:

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

where TLi is the trophic level of fish species i, $\delta^{15}N_i$ represents the $\delta^{15}N$ of fish species i, $\delta^{15}N_{pc}$ represents the $\delta^{15}N$ of primary consumers (PC; i.e. bivalve *C. glucaum*), 3.4 and 2 are the mean $\delta^{15}N$ trophic enrichment occurring per trophic level and the trophic position of the baseline organism, respectively. Commonly, the primary consumers (TL_2) are preferred to primary producers (TL_1) for evaluation of trophic levels (Cabana and Rasmussen, 1996; Vander Zanden *et al*., 1999; Vander Zanden and Rasmussen, 2001). We did not calculate the trophic levels of *C. glucaum* individuals because the stable isotopes of particle organic matter (POM) as its baseline had high variations in our sampling.

Statistical analyses

The Kolmogorov-Smirnov and Levene's tests were used to control the normality of distribution and the homogeneity of variances, respectively. Simple linear regressions were performed using ln-transformed metal concentrations in muscle vs. trophic levels. The slopes of regressions were used to determine the rate of bioaccumulation through the body size of the Caspian fishes and bivalves. One way ANOVA and Tukey test were used to assess differences among the $\delta^{13}C$, $\delta^{15}N$ values and size groups of fishes. The statistical analyses were not conducted when >50% of the observations were below the detection limit. The statistical tests were performed at the significance level of $p<0.05$ and the software SPSS version 13 for Windows was used.

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Results

According to our results, the content of C and N in samples was the lowest in bivalves, *C. glucaum* while those amounts were the highest in fish *A. chalcoides* (Table 2). The stable nitrogen isotope ratios varied among the samples from *C. glucaum* (δ^{15}N=3.5 ‰) to *S. lucioperca* (δ^{15}N=13.1‰). The stable carbon isotope ratios varied from *A. chalcoides* (δ^{13}C = -25.2‰) to *S. lucioperca* (δ^{13}C = -17.9‰) (Fig. 1). The average δ^{15}N was nearly similar for three species *R. frisii*, *L. aurata* and *A. chalcoides* (Table 2). Among fish species, *L. aurata* and *S. lucioperca* had the lowest and highest TLs; with average TLs of 3.3 and 4.2, respectively.

The statistical test showed that only δ^{13}C had significant difference (F=6.4; p<0.05; ANOVA) with small size (total length<60 mm) and big size groups (total length>200 mm) of *A. chalcoides.*

Furthermore, the δ^{15}N and TL values in the small size of *R. frisii* (total length<140mm) was more than medium (total length 141-500 mm) and big size (total length>500 mm) (F=7.3 and F=5.5, respectively; p<0.05).

Cd, Cr, Co and Ni concentrations in most of fish samples were below the detection limits; while the concentrations were detected in most samples of bivalves, *C. glucaum* (Table 1). Furthermore, the *C. glucaum* were shown to have the highest metal concentrations. Among the fish samples, however the highest mean concentration of Cu was found in *S. lucioperca* (average 9.3 µg g^{-}1), and the highest concentrations of most metals were observed in *L. aurata* (Table 1).The lowest concentrations of Mn and Fe were observed in *S. lucioperca* (average 0.7, 7.1 and 9.4 µg g^{-1} dw), respectively.

There was strong correlation between body length and body weight (Spearman's correlation coefficient = 0.99, p<0.05). Therefore, body size was used instead of body length and weight (Table 3).

An increase in trophic levels of *A. chalcoides* and *R. frisii* vs. their body size increase was observed. Generally no accumulation of metals with increase in body size was observed in muscles of all species from different trophic levels (Figs 2 and 3). Size-dependent concentrations of metals were only observed for Cr and Fe in *C. glucaum* (Fig. 2). While no significant correlation were observed between metal concentrations and fish size (also TLs) of *L. aurata*, negative significant relationships were observed between Mn and Zn concentrations and fish size for *A. chalcoides* (Fig. 3). These relationships were also observed for Cu and Zn in *S. lucioperca* and for Mn in *R. frisii*. In our study, no significant decrease in concentrations of metals was observed with increasing trophic levels in fishes (Table 3, Fig 3).

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Table 1: The biological characteristics and metal concentrations in different fishes and bivalve species collected from Anzali region (C_m; number of samples for chemical analysis, F: female, M: male, U: undefined sex, D; percentage of detected sample numbers; the average metals concentrations have been calculated from detected data, minimum values (min) have been recorded from the minimum detected data).

Species	C_m	Weight (g) Length (mm) Age (y)	Cd	Co	Cr	Cu	Mn	Ni	Zn	Fe
			Mean ± Sd (Min-Max) D	Mean ± Sd (Min-Max) D	Mean ± Sd (Min-Max) D	Mean ± Sd (Min-Max) D	Mean ± Sd (Min-Max) D	Mean± Sd (Min-Max) D	Mean ± Sd (Min-Max) D	Mean ± Sd (Min-Max) D
Percidae *Sander lucioperca*	16 (8F, 8M)	121- 549 250-365 2-3			0.77 ± 0.46 (0.36-1.47) 38%	9.3 ± 7.76 (0.22-24.7) 100%	0.77± 0.67 (0.01-0.59) 88%	1.16 6%	23.3 ± 7 (11.2-38.9) 100%	7.12 ± 5.04 (2.1-14.6) 60%
Cyprinidae *Rutilus frisii*	25 (10F, 10M, 5U)	2-1248 65-500 1-6	0.54 3%		1.4 ± 0.34 (1.1-1.7) 9%	0.22 ± 0.67 (0.12-2.55) 30%	2.6 ± 3.8 (0.26-15.7) 82%	0.81 3%	26.8 ± 13.6 (11.2-69.7) 100%	140.1 ± 198.2 (2.9-548.1) 90%
Alburnus chalcoides	20 (6F, 8M, 6U)	2.1-159 70-264 1-3	0.48 5%			1.46 ± 1.97 (0.45-5.75) 70%	3.2 ± 4.1 (0.25-14.3) 85%	0.42 5%	38.5 ± 30.4 (8.9-98.9) 80%	80.9 ± 66.3 (21-178) 94%
Mugilidae *Liza aurata*	16 (3F, M, 8U)	2.1-545 68-372 1-5		1.34 6%		3.6 ± 11.0 (0.1-40.1) 50%	3.2 ± 4.4 (0.4-12.7) 75%	1.9 ± 0.6 (1.4-2.3) 15%	27.5 ± 12.7 (11.7-51.2) 94%	217 ± 171 (61-510) 94%
Cardidae *Cerastoderma glucaum*	13	Height= 14.8-22.4 - 2-11	0.54 8%	1.44 ±0.38 (0.9-2.1) 85%	1.08±0.42 (0.44-1.62) 54%	8.94±5.52 (0.2-19.8) 100%	14.8±8.5 (5.4-35.8) 100%	15.5± 4.7 (10.4-24.9) 92%	60.4±14.2 (42.6-95.3) 100%	546.3± 430.1 (96.2-1558) 100%

Table 2: The results of isotope analysis (C_i: number of samples for isotope analysis, TL_{PC} : trophic level based on primary consumer, TL_{Pom}: trophic level based on particulate organic matter, Averages with dissimilar letters (a, b, c) specify significant difference, F: statistic value, P: significant levels.

	C_i	Length classes (mm)	$\delta^{13}C$	$\delta^{15}N$	C amount (ug)	N amount (ug)	TL_{PC}
Percidae *Sander lucioperca*	9	<300 300>	-19.97 ± 0.1 -19.96 ± 1.6 (F=0.01,*P*=0.99)	12.9 ± 0.2 12.2 ± 0.8 (F=2.6,*P*=0.15)	463.9 ± 183.1	130.5± 48.1	4.2 ± 0.24
Cyprinidae *Rutilus frisii*	13	<260 261-370 370>	-21.7 ± 0.7 -20.4 ± 1.4 -21.6 ± 10.5 (F=2.6,*P*=0.12)	8.9 ± 0.4^{a} 10.1 ± 0.9^{b} 10.5 ± 0.2^{b} (F=7.4,*P*=0.01)	326.7 ± 220.7	88.7 ± 55.0	3.4 ± 0.27
Alburnus chalcoides	8	<100 101-200 201>	-24.2 ± 1.4^{a} -21.9 ± 1.1^{ab} -21.2 ± 0.2^{b} (F=6.4,*P*=0.04)	8.6 ±1.9 10.6 ± 0.8 11.7 ± 1.0 (F=4.3,*P*=0.08)	559.1 ± 330.6	128.5 ± 68.9	3.7 ± 0.50
Mugilidae *Liza aurata*	5	<100 100>	-20.1± 0.2 -18.5 ± 0.6 (F=6.3,*P*=0.14)	8.2 ± 0.1 10.1 ± 1.3 (F=1.4,*P*=0.42)	404.3 ± 229.6	117.9 ± 71.2	3.3 ± 0.42
Cardidae *Cerastoderma glucaum*	9	Height (mm) <17 17-20 20>	-20.3 ± 0.4 -21.3 ± 1.1 -21.7 ± 1.0 (F=2.1,*P*=0.2)	4.5 ± 0.43 4.6 ± 1.6 5.0 ± 0.1 (F=0.4,*P*=0.69)	267.8 ± 121	63.9 ± 32.5	TL_{Pom} 1.41± 0.21

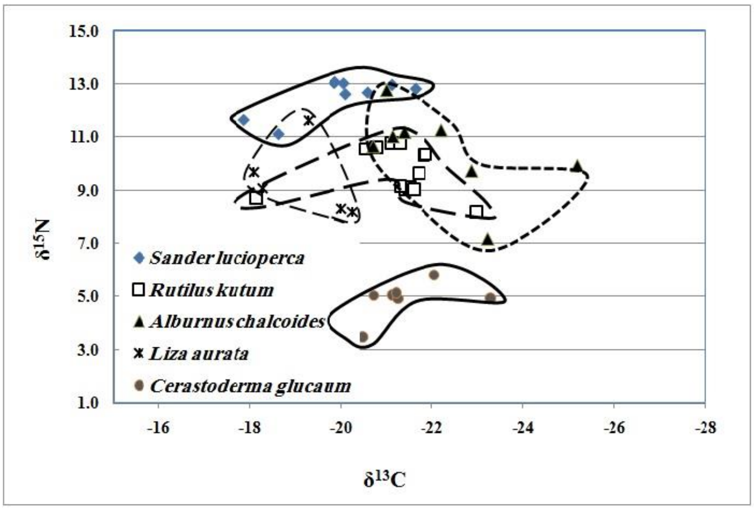

Figure 1: Distribution of carbon and nitrogen stable isotope ratios among fishes and bivalves from southwest of the Caspian Sea.

Table 3: The Spearman correlation test for dependence of Ln metal concentrations on body size (length or weight) and trophic level (* significant level <0.05).

Species		**Body size**	**Co**	**Cr**	**Cu**	**Mn**	**Ni**	**Zn**	**Fe**
Sander lucioperca	Body size				-0.65*	-0.05		-0.84*	0.97
	TL	-0.18			-0.03	0.12		-0.03	
Rutilus frisii	Body size				0.86	-0.65*		-0.39	0
	TL	0.72*				-0.57		-0.48	-0.5
Alburnus chalcoides	Body size				-0.29	-0.54*		-0.61*	-0.6
	TL	0.83*			-0.8	-0.25		-0.64	-0.2
Liza aurata	Body size				0.14	-0.5		-0.44	0.1
	TL	0.67			0.6	-0.86		0.46	0.32
Cerastoderma glucaum	Body height		0.11	-0.93*	-0.19	-0.55	0.22	0.08	-0.63*
	TL	0.49	-0.11	0.4	0.5	-0.11	0.05	0.03	-038

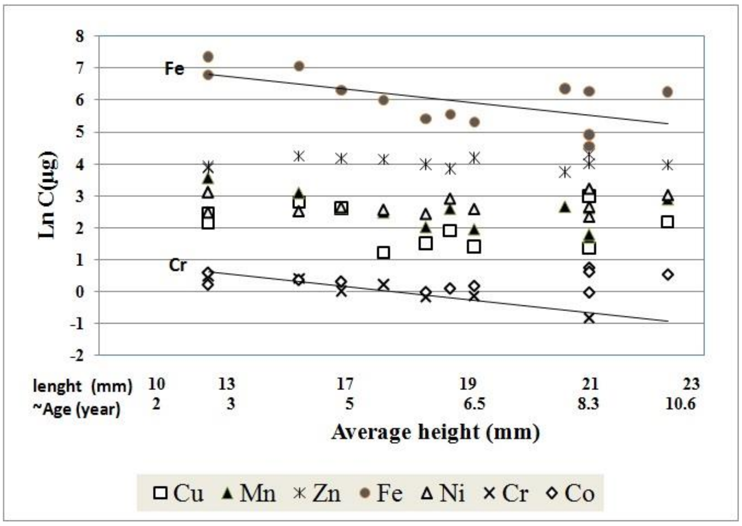

Figure 2: Regression between Ln metal concentration on height and trophic levels of bivalves (significant differences have been showed by lines).

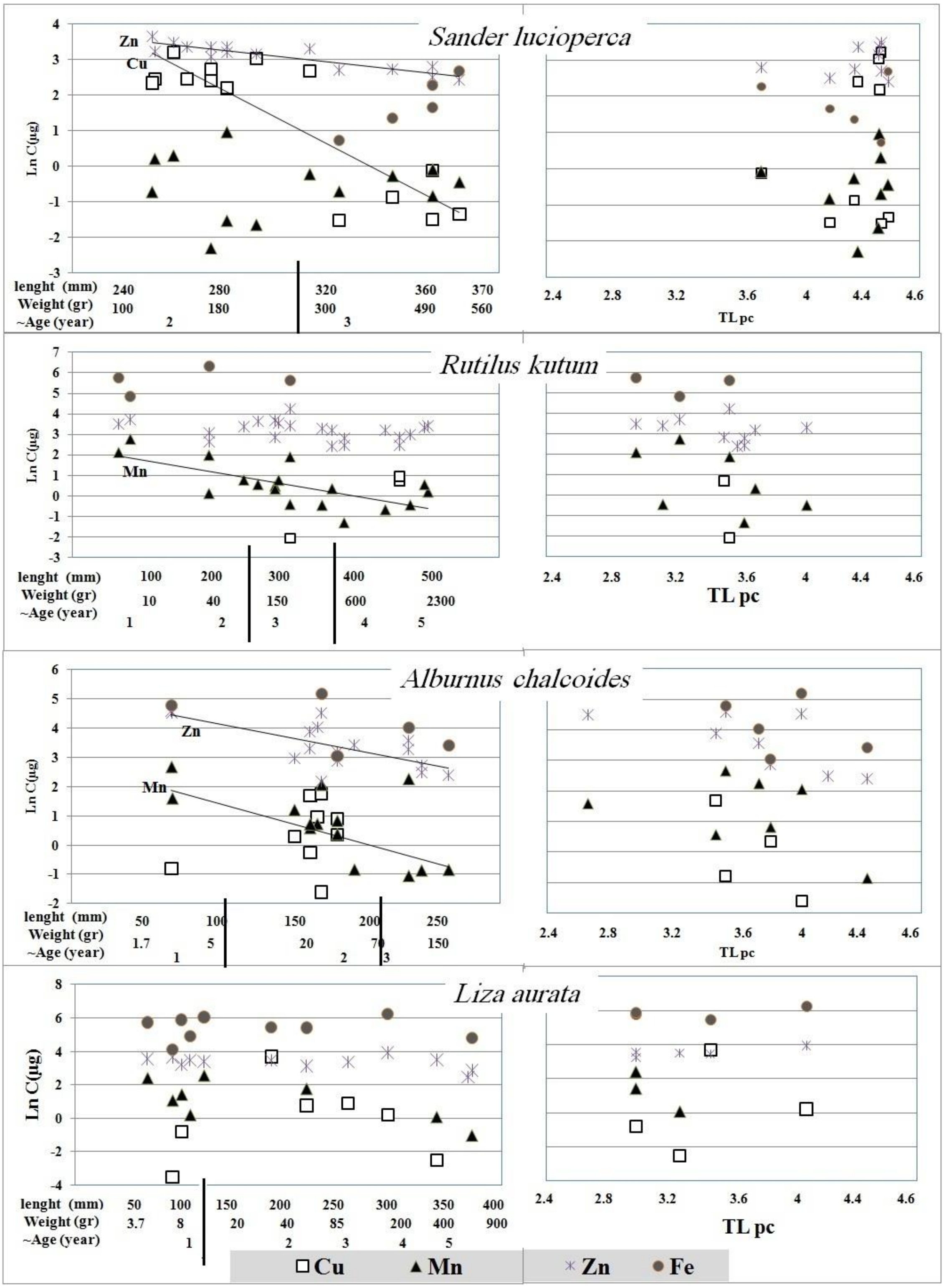

Figure 3: Regression between Ln metal concentration on body length and trophic levels of fishes (significant differences slopes have been showed by lines; vertical lines in horizontal axis indicated the body size classes).

Table 4: Variations of metals concentrations in *Liza aurata* and *Rutilus frisii* in the Southern Caspian Sea as reported in previous investigations (*based on wet weight).

Species	Cd	Co	Cr	Cu	Mn	Ni	Zn	Fe	References
Liza aurata	ND		0.43						Pazooki *et al*., 2009
	0.04					0.12			Fallah *et al*., 2011*
	0.02								Shahryari *et al*., 2010
	0.02			1.3			3.4		Ardalan *et al*., 2012
	0.03		0.08						Tabari *et al*., 2010
	0.63	ND		10.85		`0.41	217.8	125.6	Varedi, 2011
Rutilus frisii	0.001	0.009	0.33	1.01	0.45		17.2		Anan *et al*., 2005
				2.7			22.3		Foroughi Fard *et al*., 2008
	ND			2.76			15.4		Monsefrad *et al*., 2012
	0.17					0.52			Fallah *et al*., 2011*
	0.11								Hoseini and Tahami, 2012
	0.02								Shahryari *et al*., 2010
	0.325		ND	1.68	0.78	2.65	0.63	5.6	Eslami *et al*., 2011*
	0.04		0.06						Tabari *et al*., 2010
	ND	ND		0.013		ND	0.4	0.08	Varedi, 2011

ND: not detected

Discussion

The study showed that the concentrations of Mn, Zn and Fe in *Rutilus frisii* and *Liza aurata* were higher than those reported in previous studies (except Cu level; Tables 1 and 4). It might be related to temporal rise of the Caspian pollutants. According to Karpinsky (1992) the concentrations of metals in sediments of the Caspian Sea have shown high temporal increases. There are a few investigations showing variations in pollutant concentrations in the southern Caspian Sea. Among them, several authors have reported high variations in metals concentrations of water and substrate (Laloei, 2001; Parizanganeh *et al.*, 2007; Hashemian kafshgari, 2008; Sohrabi *et al.,* 2010).

While only Cu concentration had the highest concentrations in piscivorous *S. lucioperca,* no significant difference in metals was observed in deposit feeder *L. aurata* and benthivorous (*R. frisii* and *A. chalcoides)* (Table 1). According to Goodyear and McNeill (1999) the animals took up Zn in direct proportion to levels found in sediments while Cu had similar uptake rates from the water and sediment. Therefore the high concentration of Cu in *S. lucioperca* might be related to its different feeding habits.

The low concentration of Cr and Cd, similar to what found in previous studies (Tables 1 and 4), might be related to their concentrations in sediments. The local sources might be responsible for metal pollution in the Caspian Sea. Similarly, Anan *et al.* (2005) found higher concentrations of Co, Cd in fishes from our study area than that found in other parts of the Caspian. Also higher concentrations of metals such as Cd were observed in the central and southern part of the Caspian Sea than those in the northern sector (de Mora *et al.*, 2004) because of their rich natural background resource.

In our investigated species, the depleted and enriched $\delta^{15}N$ values can be due to their diets; i.e. deposit feeders and piscivorous, respectively (Table 1, Fig. 1). *A. chalcoides* has been known as a zooplankton feeder (Abbasi *et al.*, 2004) but its mean TL value is relatively higher than benthivorous species *R. frisii* (Table 2). Comparatively, *R. frisii* seems to have longer-distance movements and probably feeds on depleted $\delta^{15}N$ sources; while *A. chalcoides* exists in inshore littoral areas where the eutrophication and subsequently the trophic base line is higher than offshore regions. The $\delta^{15}N$ values of primary consumers and other organisms in coastal areas would be increased after eutrophication and upward N loading phenomena (Cabana and Rasmussen, 1996; Martinetto *et al.*, 2006). Asante *et al.* (2008) also found the higher $\delta^{15}N$ in fishes from shallow water rather than deep water. Furthermore, a broad range of $\delta^{13}C$ values were observed in *A. chalcoides* (Fig. 1). Hobson *et al.* (2002) reported that the high variation in $\delta^{13}C$ of benthivorous species might be related to the broad range of $\delta^{13}C$ in benthic compared to pelagic organisms. A higher $\delta^{13}C$ value observed in *L. aurata* (Fig. 1) also might be due to their diet that mostly contains the substrate invertebrates and the meiofauna (Ghadirnejad, 1996).

The significant slopes of metals concentrations, the fish size and the TLs showed that the metals do not bioaccumulate, but possibly biodilute by fish size and the TLs (Table 3, Fig. 2). Similarly, correlation between $\delta^{15}N$ and metals concentrations in fishes was not observed in other investigations (Campbell *et al.*, 2005; Asante *et al.*, 2008; Ikemoto *et al.*, 2008; Zhang and Wang, 2012).

A significant positive correlation between TLs and fish size was only observed in Cyprinid species (Table 3). It is probably related to change or variety of feeding habits for different sizes; while the Percidae and Mugilidae samples are known as piscivorous and deposit feeders, respectively. Atherin and kilka followed by Mugilidae were found to be the main food items of *S. lucioperca* (Abbasi *et al.*, 2004). According to Vinagre *et al.* (2011), there was no correlation between the Carbon and Nitrogen stable isotopes of muscles and body size. It seems, there is a large range between $\delta^{13}C$ and $\delta^{15}N$ values in fish particularly *A. chalcoides* (Fig. 1) due to different food items size. The findings showed, the $\delta^{13}C$ value in

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the small size of *A. chalcoides* was significantly lower than in big sizes of the fish (F=6.4; $p<0.05$; Table 1). The small size of *A. chalcoides* might feed on the micro-zooplankton while the big size of this fish feed on the crustacean and macro-planktons (Abbasi *et al*., 2004).

The low significant $\delta^{15}N$ (F=7.3; $p<0.05$; Table 1) in the small size groups of *R. frisii* as compared with the big size groups could be related to variations in their diets. Additionally, Zarinkamar (1996), Abdolmaleki *et al*. (2009) and Afraei Bandepei *et al*. (2009) noted that the big size groups of *R. frissi* feed on bivalves, while the diet of small size groups was crustacean in the Caspian Sea.

Although there were a strong relationship between TLs and body size in Cyprinid species (Table 3), there were no significant slopes between TLs and the Ln concentrations of metals (Fig. 2).

A significant negative relationship was found between the concentrations of Zn vs. body size for most fish species (Table 3). However, in the biomagnification of Zn documented in a few investigations on food web (Quinn *et al.*, 2003; Campbell *et al.*, 2005), there was also a significant negative correlation observed between $\delta^{15}N$ and Zn concentration (Yoshinaga *et al.*, 1992).

The negative relationship between body size and metal concentration in muscle (Table 3) has been previously reported in many Caspian fishes (Anan *et al.*, 2005; Pourang *et al.*, 2005; Pazooki *et al.*, 2009). The relationships between metals concentrations in the muscle and length have been widely documented by several investigators. Some contradictory results have been discussed for fishes in the world by Pourang *et al.* (2005). Growth (body weight, length and age) dependent decreases in metals concentrations have been reported for many fish species particularly for Zn, Cu, Cd and Mn in muscle tissues (Anan *et al.*, 2005). Significant negative correlations were found for Co, Zn, Cu in *Rutilus caspicus* (Anan *et al.*, 2005). While Hoseini and Tahami (2012) found a significant relationship between Cd concentrations in muscle and *R. frisii* size, no significant relationship was observed for Cu and Zn concentrations by Foroughi Fard *et al.* (2008). Pourang *et al.* (2005) did not observe any age related differences in metal concentrations in different species; and only the concentration of cadmium had a negative relationship with weight of the samples ($p=0.038$). A significant positive relationship was found between bioaccumulation factors and fish age in *Liza saliens* (Fernandes *et al.*, 2007).

Various environmental and biological factors, nourishment source and seasonal changes affect the differences in metal bioaccumulation (Agusa *et al.*, 2004; Wang and Rainbow, 2008). The findings showed, there was no bioaccumulation of metals in *S. lucioperca*, *A. chalcoides* and *L. aurata*, so no significant differences

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were observed between $\delta^{15}N$, TLs and the fish sizes.

The metabolic rate and dilution of metal during growth period can be responsible for this relationship; as smaller fish have higher metabolic rates and are able to accumulate metals, via food and water, more rapidly than larger fish (Anan *et al.*, 2005). The *R. frisii* had a significant difference in $\delta^{15}N$ among their different body sizes which could be related to difference in metal concentration in their food diet.

The mean metals concentrations in muscles were lower than the provisional tolerable daily/weekly/monthly intake of the metals, as established by the United States Environmental Protection Agency, European Food Safety Authority, Ministry of Agriculture Fisheries and Food, and the FAO/WHO (the values presented by Fallah *et al.*(2011), Zhang and Wang (2012), Mashroofeh *et al.* (2013)).

The metals concentrations in the muscles of fishes were lower than those reported in the previous studies. These concentrations could not pose any threat to human health. In addition, no bioaccumulation was observed for metals in different trophic levels of *R. frisii* in the Caspian Sea. It is suggested that monitoring of metals in aquatic organisms and the Caspian environment should be performed to further discover those trends.

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