TRACE ELEMENTS IN WHITING *MERLANGIUS MERLANGUS* AND ITS PARASITE *HYSTEROTHYLACIUM ADUNCUM*: ROLE OF AGE, SIZE AND PARASITISM ON THE HOST

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Abstract. We measured the concentrations of the trace elements B, Mn, V, Co, Cr, Ni, Cu, Zn, As, Se, Mo, Pb and Cd in whiting *Merlangius merlangus* infested or non-infested with the intestinal helminth *Hysterothylacium aduncum*, as well as the concentrations in *H. aduncum* itself using inductively coupled plasma mass spectrometry (ICP-MS). The levels of most trace elements did not differ between the tissues of infested and non-infested fish. The tissues and parasites of younger fish (<2.1 year old), however, carried higher amounts of trace elements than those of older individuals. *H. aduncum* accumulated all trace elements in greater amounts, up 255 folds, than any fish tissues tested. These results support the idea that the higher metabolic activity of younger fish may lead to higher amounts of trace element accumulation in their tissues. Moreover, trace element accumulation is not cumulative as fish grow, and fluctuates during various life cycle of fish.
Keywords: *trace elements, Merlangius merlangus, Black Sea, parasite, fish.*

Introduction

Trace element accumulation in seafood is a major environmental concern (Gunkel, 1994), particularly in closed seas under heavy anthropogenic pollution pressure such as the Black Sea. Trace elements, required by the organisms in minute amounts, are considered potentially environmental toxic substances if their levels are higher than biologically acceptable levels. While the majority of trace element pollution comes from parent rocks and metallic minerals, accumulation of trace elements from effluents in contact with agricultural and industrial activities, as well as with shoreline urban development has intensified in the last decade (Adriano, 2001). Therefore, an assessment of trace elements accumulating in ailments through seafood is needed to be able to initiate and implement proper management strategies.

Bioaccumulation of trace elements in the indicator model organisms may provide an insight into the health of the ecosystem. Since not all bioindicator organisms behave biologically the same for a given trace element, an urgent evaluation is required to determine their robustness as models for trace elements accumulation in an ecosystem. Though various fish tissues have been proposed for pollution monitoring in aquatic ecosystems (Eira et al., 2009), the bioamplified trace element levels in parasites could make these organisms a better trace element pollution indicator than their hosts. Several studies strongly indicate higher levels of trace element accumulation in intestinal

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acanthocephalans in comparison to the levels in their fish hosts (Gabrashanska and Nedeva., 1996; Galli et al., 1998; Sures et al., 1999). However, many questions regarding the accumulation of trace elements in a host-pathogen interaction remain to be answered. *Anguilla carassus*, found in the intestines of the European eel *Anguilla anguilla*, did not accumulate lead as the acanthocephalan *Paratenuisentis ambiguous* (Sures et al., 1999; Sures et al., 1994; Zimmermann et al., 2004). As accumulating evidence suggests, the bioaccumulation of trace element dynamics in parasites likely depends on the host, trace element type, and environments. Gabrashanska and Nedeva (1996) interestingly reported that the trace elements Cu and Zn, are higher in, parasitized fish tissues compared to unparasitized fish, assessing that trace elements accumulation in fish is biased by parasite infection. Furthermore, because infested fish are more prone to trace element toxicity (Boyce and Yamada, 1977; Pascoe and Cram, 1977), other such factors as tissue types, age or sex of fish are to be evaluated to understand their influence on the bioaccumulation of trace elements, both in infested and non-infested fish. Moreover, it is still not clear whether high accumulation of trace elements in parasites indicates lower retrospective exposure for the host in comparison to non-infested fish.

The whiting *Merlangius merlangus*-nematode *Hysterothylacium aduncum* host-parasite constitutes an excellent candidate model system for monitoring the accumulation of trace elements in the Black Sea. The fish is a non-migratory species in the Black Sea, allowing for its easy sampling in its local environment at any time of the year. In addition, the whiting is a bottom dwelling fish and therefore possibly exposed to trace elements more than pelagic fish. Moreover, being a carnivor species preying on all fish species including its own, there is a high prevalence of intestinal parasitic nematodes in adult individuals enabling satisfactory levels of sampling adult nematodes from each fish.

Here, we aimed to assess whether trace element concentrations in whiting infested with *H. aduncum* or free of the parasite differed. Moreover, we also evaluated the effects of size and sex of the fish host as a factor for bioaccumulation. As we hypothesized that parasite- infested fish accumulated more trace elements due to parasite induced enhancement of the metabolic activity, we compared trace element between sexes and in various age classes of parasitized and non-parasitized fish, as well as with the intestinal parasites themselves. This study stand as a model for monitoring and evaluating trace element pollution in the Black Sea.

Material and Methods

Fish and sampling

Whiting *Merlangius merlangus* both infested and non-infested with the nematode *Hysterothylacium aduncum* (nematoda: anisakidae) were collected (n = 288) using dip-nets during December and January, 2011 in the Surmene Bay, Trabzon Province, Turkey, in the south-eastern Black Sea (40°55' N; 40°11' E) (*Table 1*). The sampling was carried out at the same place in two consecutive months as the whiting is a local, non migratory species. After measuring length (cm) and weight (g), the samples were sorted by sexes and three length (total length) classes: 1) smaller than 15, 2) between 15-17 cm and 3) bigger than 17 cm. Subsequently, these length classes were transformed, by the von Bertalanffy equation, to age classes as previously described

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(Altuntas and Ogut., 2010) using the data referring to the same species, in the same region of Turkey (Ciloglu et al., 2001).

Table 1. *Sampling scheme of whiting* M. merlangus *for age groups, sexes, and tissues*.

Age Class	Sex	Tissue	Number of Pools	Fish/pool	n
< 2.1	Male	Sperm Duct	3	10	93
		Liver	3	9	
		Muscle	3	10	
		Gills	3	11	
	Female	Gonad	3	8	84
		Liver	3	7	
		Muscle	3	7	
		Gills	3	7	
2.1-2.9	Male	Sperm Duct	3	8	36
		Liver	3	7	
		Muscle	3	8	
		Gills	3	8	
	Female	Gonad	3	13	52
		Liver	3	13	
		Muscle	3	13	
		Gills	3	13	
>2.9	Male	Sperm Duct	2	1	5
		Liver	2	1	
		Muscle	2	1	
		Gills	2	1	
	Female	Gonad	3	5	18
		Liver	3	5	
		Muscle	3	5	
		Gills	3	5	

Tissue and intestinal parasite sampling

All whiting sampled in the study was dissected to remove the viscera and to obtain tissue samples for trace element analysis (*Table 2*). About 0.5 g of muscle, liver, gills, gonad, refers to ovaries (female) or sperm duct of fish from each stratification were pooled (five to eight of the same tissue/pool) and analysed for metal content. The digestive tracts were then cut longitudinally to determine the number of adult nematode. Note that all nematodes present in the digestive truck are adults, L5 stage nematodes, therefore "infested fish" refers to fish having adult nematodes in their intestines. Generally, infested fish have one to five adult nematodes in their digestive tract. In a parallel sampling, analyses of the 15 individual digestive tract nematodes cleared by glycerin and photomicrographed all were identified as *H. aduncum* according to the keys created by Skryabın (1969).

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***Table 2**. Sampling scheme of the intestinal parasite H. aduncum from its host M. merlangus.*

Age Class	Infested	Sex	Number of Pools	Fish/pool	n
< 2.9	Y	Male	3	4	265
		Female	3	4	
	N	Male	4	29	
		Female	4	31	
>2.9	Y	Male	0	0	23
		Female	1	1	
	N	Male	2	3	
		Female	3	6	

Measurement of trace elements

All fish tissues and parasites were freeze-dried until constant weights were acquired. After homogenization, about 0.5 g of dry tissue was digested in 7 mL nitric acid+1 mL hydrogen peroxide in a microwave digestion unit (Milestone Ethos 1, HPR-FO-17). Then, the samples were further diluted to 25 mL using deionized water. The same procedures were also applied to the blank samples. The amounts of trace elements B, Mn, V, Co, Cr, Ni, Cu, Zn, As, Se, Mo, Pb and Cd were measured using a high performance inductively coupled plasma mass spectrometer (Bruker 820 ICP-MS) operated in collision reaction interface (CRI) mode to overcome polyatomic interferences. The accuracy and reliability of the analyses were assured using certificated reference material: DORM 3 (Fish protein). Internal standards having Li, Sc, Y, In, and Tb (50 ppb (ug/L) were used as reference. The calibration standards and the samples were matrix-matched. Reference material and their recovery are listed in *Table 3*.

***Table 3.** Metal concentrations (µg g-1 dry wt) in certified reference material (DORM-3).*

Metals	Certified value	Observed value	Recovery (%)
Cr	1.89±0.17	[a]1.85±0.19	97.88
Ni	1.28±0.24	1.24±0.22	96.88
Cu	15.5±0.63	14.8±0.43	95.48
Zn	51.3±3.10	49.18±0.89	95.87
As	6.88±0.30	6.70±0.36	97.38
Cd	0.29±0.02	0.28±0.04	96.55
Hg	0.38±0.06	0.35±0.06	91.62
Pb	0.40±0.05	0.38±0.05	95.00

Values indicate the average value ± SE

Parasites belonging to the oldest age group (>2.9 year old), and a combined sample of parasites from the smallest age group and middle age group fish were assayed to determine the rates of trace elements in a unit of sample, using the same procedures described above except for the digestion, which was carried out in 1mL nitric acid at room temperature because of the small pool size of parasites.

Statistical analysis

Metal concentrations in parasitized and unparasitized fish were compared using a two-way ANOVA test by taking tissues and age classes, or tissues and sexes, into consideration as variables. Moreover, using the same two-way ANOVA approach, we also compared the amounts of trace elements in different tissues of male and female or age groups ignoring the infection status. The levels of trace elements in parasites were compared using chi-square test. *P*-values less than 0.05 were considered statistically significant.

Results

Influence of age and sex on accumulation of trace elements in M. merlangus

To assess whether different whiting tissues accumulated trace element in a similar fashion, we quantified B, Mn, V, Co, Cr, Ni, Cu, Zn, As, Se, Mo, Pb and Cd in muscle, liver, gills and gonad (ovaries) or sperm ducts by ICP-MS. Tissues had statistically different levels of trace elements, with the exception of Cr and Ni (One-way ANOVA, P<0.05, *Figs. 1 and 2*). Accumulation of Pb, B, V, Mn, Co and Se in the gills, Mo and Cd in the liver, and Cu and As in the gonad/sperm duct and liver were significantly higher compared to the other tissues tested (P<0.05). Therefore, our analysis of these thirteen trace elements showed that fish tissues accumulated trace elements in different quantities.

To determine whether age was a factor influencing trace element accumulation, we divided our fish samples into three age classes. The youngest age class (<2.1 year old) had significantly (P<0.05) higher amounts of trace elements than the oldest fish (>2.9 year old) (*Figs. 1 and 2*). The age effect was mainly visible for the amounts of Mn, V, Ni, Mo and Cd in the youngest fish group, whereas Pb was the only trace element highly concentrated in the gills of the oldest age class (*P*<0.05).

Sexes of fish also had an impact on the accumulation of some of trace elements in whiting (*Fig. 2*). Co, Zn, As and Se, were present at higher levels in gonads (ovaries) than in other tissues, whereas the concentrations of trace elements in the tissues of male and female whiting were not significantly different from each other (*P*>0.05).

Influence of parasite infestation on accumulation of trace elements in the host

Twenty-four out of 288 of the whiting sampled for this study were infested with the adult nematode, *H. aduncum*. We therefore assessed whether the presence of parasite influenced accumulation of trace elements in the whiting host. The amounts of the trace elements Ni, Pb, Mn, Co and Mo were significantly higher in the infested fish than that in non-infested fish (P<0.05; *Figs. 1 and 2*). Furthermore, the younger infested whiting carried higher amounts of trace elements compared to individuals of the other two age classes.

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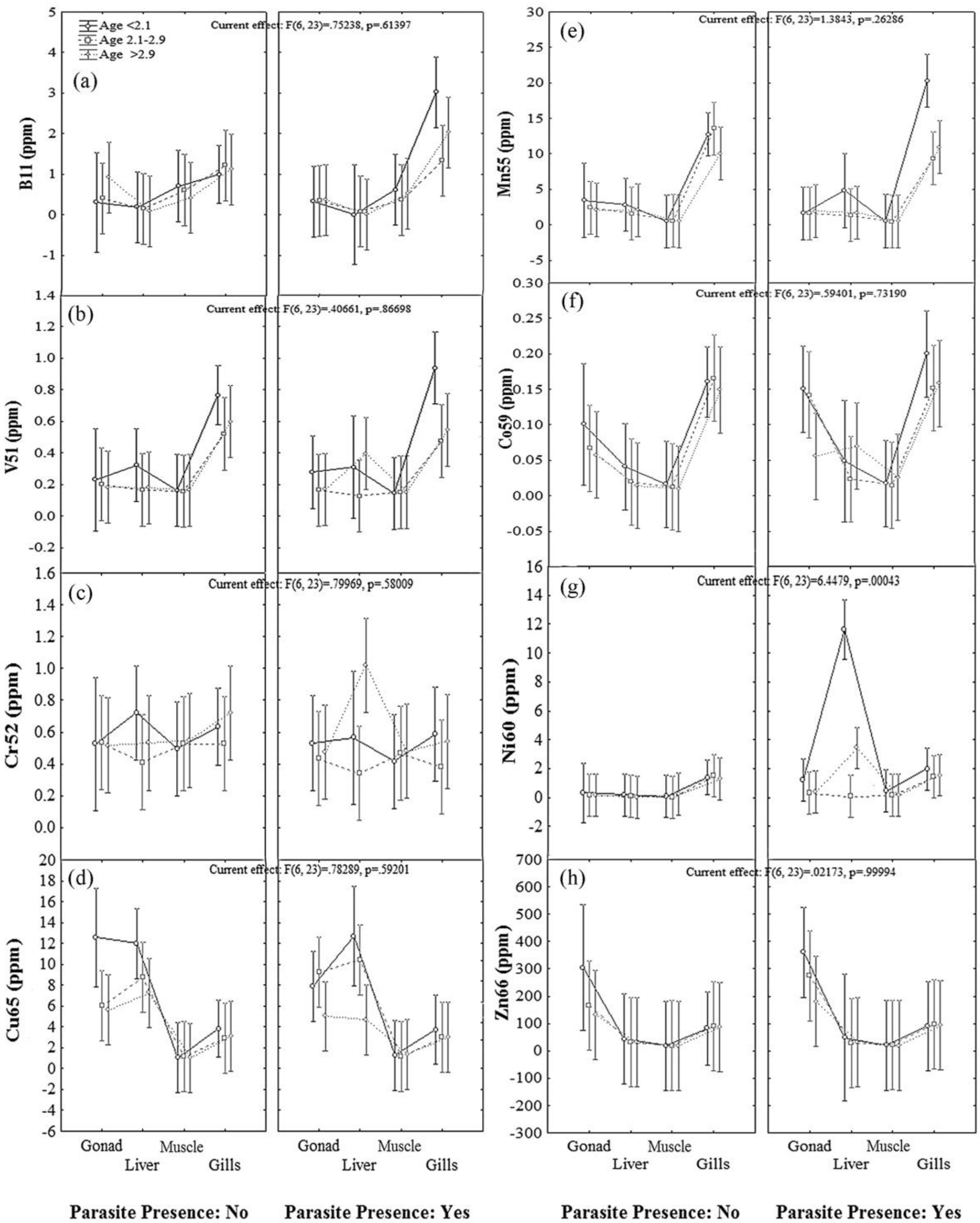
This was most evident for the accumulation of the trace element Ni: the younger age class presented significantly higher amounts of Ni in the kidney (P=0.0043; *Fig. 1g*) compared to the other two classes. Moreover, the levels of Ni in the kidneys of mid-age class whiting were higher than that of the oldest age class fish (P<0.05).



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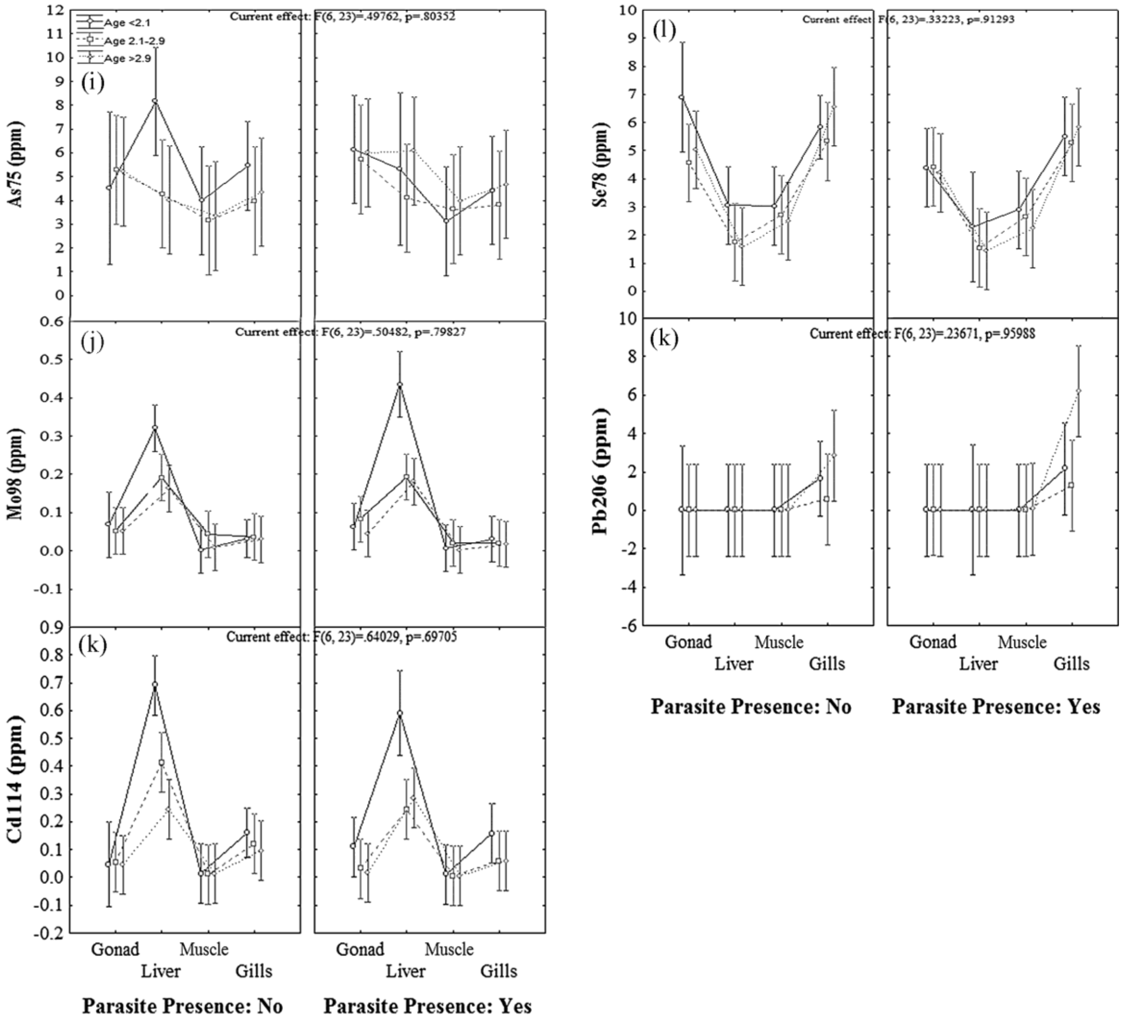

Figure 1. *Concentrations of trace Elements in various tissues of whiting M. merlangus in three age classes (<2.1, 2.1-2.9, and >2.9) with and without the endoparasite H. aduncum sampled in the south-eastern Black Sea. Bars denote 95% confidence intervals.*

The concentration of B was similar in both infested and non-infested fish in the two older classes (P>0.05), whereas they were higher in the gills of young whiting (P<0.05; *Fig. 1a*). We measured a similar trend for Mn and V (*Fig. 1e,1b*). Pb amounts in infested fish were also higher than those of non-infested fish. However, the amounts of Pb in gills were significantly higher than Pb in other tissues in old whiting (P<0.05; *Fig. 1k*).

The concentrations of Co and Zn were significantly higher in the gonad (ovaries) of infested fish than that of non-infested fish (P<0.05; *Fig. 2b*). Both As and Mo concentrations were higher in the kidneys of male fish than that of female fish (*Fig. 2c*). The rate of As was significantly higher in the sperm duct of infested fish than that in the ovary of infested whiting (P<0.05).

Trace elements in the endoparasite H. aduncum

Parasites carried higher amounts of trace elements than any host tissue tested. For example, Cu amounts in the parasites of the younger group whiting were 3 times, 2.5 times times, 19 times and 7 times higher than the average trace element rates found in

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host tissues; gonad, liver, muscle and gills respectively (*Table 4*). Moreover, various tissues presented different odds of the level in the parasite to the level in the tissue. The amount Cd was 255 times higher in the same group of parasites than that in the muscle of the respective hosts, whereas it was 49 times, 6 times and 22 times of that from ovaries, liver and gills respectively.. Analysis of intestinal parasites from young (<2.9 year old) and older (>2.9 year old) whiting also showed that the trace element amounts in parasites were higher in the nematodes found in younger fish, except for the levels of Cd and Co (*Table 4*). The differences of average trace elements in mid-age class, younger, and older fish were statistically higher for the eight trace elements (B,V, Cr, Mn, Cu, Zn, As and Se) tested (*P*<0.05; *Figures 1 and 2*). Moreover, parasites in the younger fish always presented higher rates of trace elements.

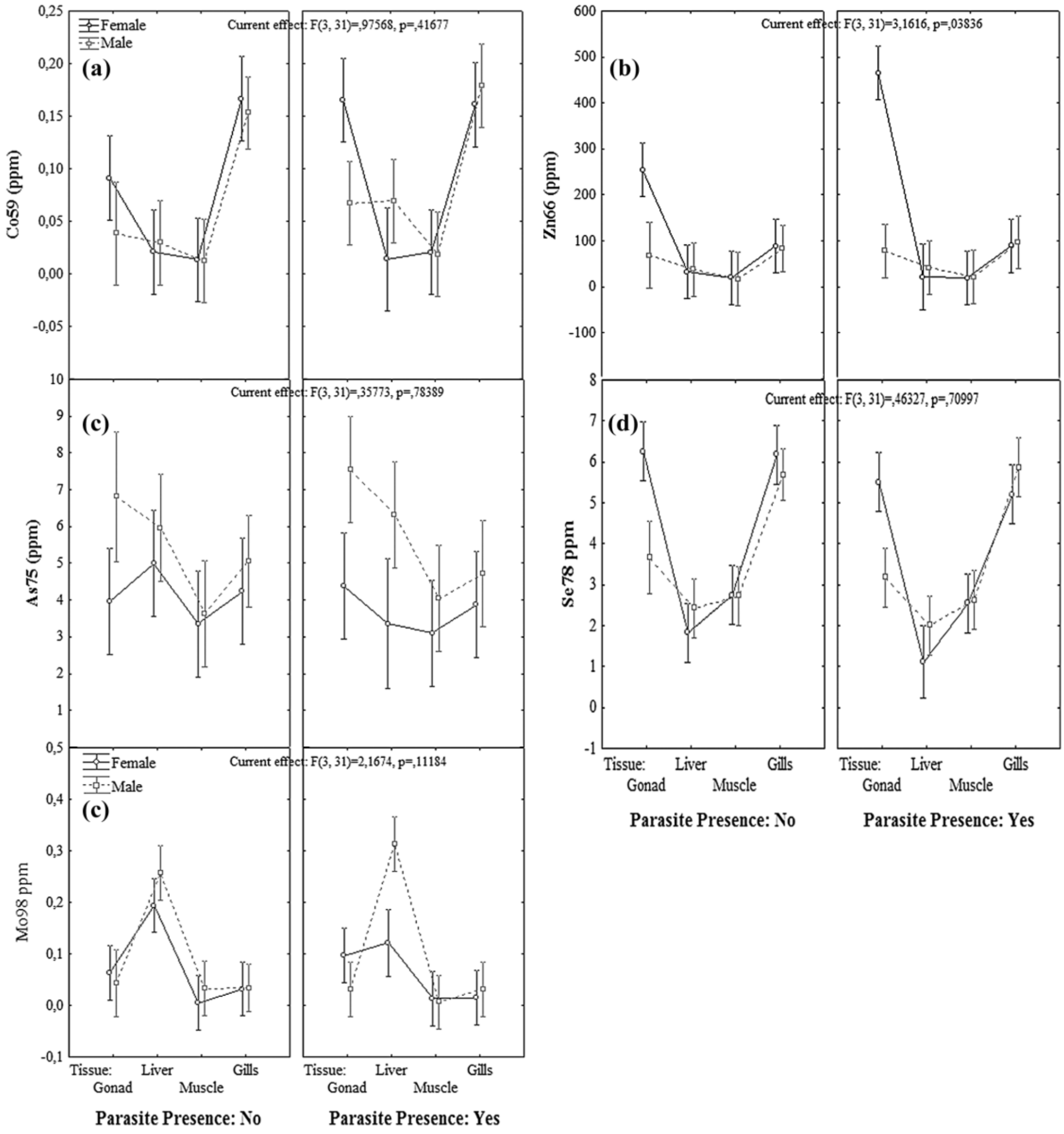

Figure 2. *Concentrations of trace Elements in various tissues of male and female whiting M. merlangus with and without the endoparasite H. aduncum in the south-eastern Black Sea. Bars denote 95% confidence intervals.*

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Table 4. *Trace element levels measured in endoparasite H. aduncum harvested from whiting M. merlangus aged less than 2.9 year old or more than 2.9 year old. Chi-square tests were used to compare the levels of trace elements in parasites collected from different age classes. The parasites from these two age classes were tested separately. . All parasites were grouped according the host's age and sexes due to considerable small size of the parasite. Values for amount of trace elements in host tissues are represent as average values ± standard The same letters refer to the groups statistically the same deviation*

	Amount of Trace Element in Parasites (micrograms per g dwt)			*Amount of Trace Element in Host Tissues (micrograms per g dwt)*			
Trace Element	*Age class < 2.9*	*Age class > 2.9*	*P-Value*	*Gonad*	*Liver*	*Muscle*	*Gills*
B	*8.3*	*5.6*	*0.001*	0.46 ± 0.11^a	0.09 ± 0.02^a	0.54 ± 0.05^a	1.57 ± 0.29^b
V	*3.25*	*2.75*	*0.361*	0.20 ± 0.01^a	0.25 ± 0.05^a	0.16 ± 0.01^a	0.65 ± 0.07^b
Cr	*10.1*	*8.05*	*0.030*	0.50 ± 0.02	0.60 ± 0.11	0.49 ± 0.02	0.57 ± 0.03
Mn	*20.75*	*14.95*	*0.000*	2.07 ± 0.83^a	2.17 ± 0.83^a	0.51 ± 0.79^a	12.82 ± 0.76^b
Co	*2.4*	*2.6*	*0.689*	0.10 ± 0.01^a	0.04 ± 0.01^b	0.02 ± 0.01^b	0.16 ± 0.01^c
Ni	*2.9*	*1.95*	*0.054*	0.43 ± 0.56	1.75 ± 0.56	0.16 ± 0.54	1.50 ± 0.52
Cu	*22.55*	*16.9*	*0.000*	7.28 ± 0.74^a	8.99 ± 0.74^a	1.16 ± 0.71^b	3.30 ± 0.68^b
Zn	*384.15*	*282.9*	*0.000*	230.26 ± 27.75^a	34.61 ± 27.75^b	19.63 ± 26.57^b	88.77 ± 25.52^b
As	*18.65*	*16.6*	*0.122*	5.57 ± 0.44^a	5.3 ± 0.44^a	3.53 ± 0.42^b	4.52 ± 0.40^b
Se	*24.25*	*11.85*	*0.000*	4.74 ± 0.27^a	1.91 ± 0.27^b	2.66 ± 0.26^b	5.73 ± 0.24^a
Mo	*0.95*	*0.8*	*0.612*	0.06 ± 0.02^a	0.23 ± 0.02^b	0.015 ± 0.06^b	0.03 ± 0.015^a
Cd	*2.45*	*2.55*	*0.841*	0.05 ± 0.032^a	0.40 ± 0.032^a	0.01 ± 0.03^a	0.11 ± 0.03^b

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Discussion

In this study, we investigated whether bioaccumulation of trace elements in whiting *M. merlangus* was influenced by sex, age or by the presence of the intestinal parasite *H. aduncum*. The age of the host appeared to be the principal factor in the bioaccumulation in both tissues of whiting and their respective intestinal parasites. Moreover, the amounts elements were significantly higher in the intestinal parasites than those in the tissues of the host. Our results indicate that the whiting – nematode model could be used for studying dynamics of trace element accumulation in respect to the age, sex and host tissues and be used for biomonitoring of trace element pollution in the Black Sea.

While the amounts of five trace elements (Co, Ni, Mo, As and Cd) in younger and older fish was the same, B, V, Cr, Mn, Cu, Zn and Se levels were significantly higher in younger fish, probably due to the differences in metabolic activity of younger and older fish. Older fish was expected to have higher levels of trace elements indicating retrospective exposure from water. However, our results show that younger fish have higher levels of trace element accumulation. It should be noted here that according to these results surrounding water column seems to be not the biggest source of trace elements. Other reasons such as metabolic rate differences, food quality or foraging shifts of whiting in their life cycle could be responsible for this difference. Younger fish have higher weight specific metabolic rates (mg $O_2 \cdot kg^{-1} \cdot h^{-1}$), such as consumption of oxygen at a given time, than older fish (Sims, 1996). Furthermore, trace elements accumulation increases fish metabolic rate (Vinodhini and Narayanan, 2008). Our result also showed that infested young fish accumulated higher amounts for trace elements that infested old fish: it is possible that parasite induced enhancement of the metabolic activity may make the fish host more prone to environmental toxicity (Boyce and Yamada, 1977; Pascoe and Cram, 1977), another piece of evidence suggesting that the metabolic rate could be related to trace element accumulation. Higher metabolic rate will result in higher energy and oxygen demand, leading to more exposure to trace elements by the elevation in the gill ventilation rates and feeding to comply extra energy costs. The amounts of trace elements in parasites collected from younger fish were also higher from those in older fish, indicating that the rates of accumulation is different during their life history. Accumulation and excretion of trace elements could fluctuate seasonally (Retief et al., 2009). Therefore, further study is necessary to verify potential seasonal effects.

Our study showed that infested fish had higher levels of trace elements comparing to non-infested ones. This is in contrast with previous report showing that infested fish have lower levels of metals compared to non-infested fish from the same area (Azmat et al., 2008; Morsy et al., 2012). It is possible to argue that the amount of trace elements is expected higher in infested fish comparing to non-infested fish: since parasites compete with their hosts for nutrients, this would also lead to sharing of trace elements between host and parasite. Therefore, the amount of metals in the non-infested fish is expected to be higher than that in non-infested fish.

Higher amounts of trace elements in parasites compared to the tissues of its host is reported in sea bream (*Sparus aurata*), a culture fish in the Mediterranean Sea (Dural et al., 2011). In our study, in accordance, we found that Cr and Co were markedly higher in parasites than that in the host tissues. Morsy et al. (2012) reported that the amount of Ni and Mn were significantly higher in parasites compared to the tissues of the host sea

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bass *Dicentrarchus labrax.* Similarly, the trace metals Pb, Cd, Hg, As, Zn and Fe found higher in parasites comparing to the concentrations in tissues of their host *Liza vaigiensis* (Azmat et al., 2008). Our data therefore confirm that *M. merlangus* and *H. aduncum* have a trace element accumulation pattern similar to other host-parasite models reported (Azmat et al., 2008; Eira et al., 2009; Sures, 2003).

All trace elements accumulated at higher levels in parasites than in tissues of host, in accordance to the findings of some researchers (Azmat et al., 2008; Eira et al., 2009; Sures, 2003). Most studies of parasite bioaccumulation have used acanthocephalans as model organisms. As reviewed by Sures (2003), acanthocephalans cannot synthesize their own fatty acids and cholesterols, therefore they depend on their hosts intestinal lumen to obtain them. Bile acids present in host intestine facilitate formation of organo-metallic complexes, more readily available for absorption by the host and parasites (Sures and Siddall 1999; Zimmermann et al. 2003). Similar studies have been also performed with nematodes as well. Nematode parasites *Echinocephalus* sp. and *Ascaris* sp, presented several folds of trace elements in comparison to tissues of their host harbor porpoise (*Phococena phocoena*) (Azmat et al., 2008; Szefer et al., 1998).

In summary, our study shows that the age of whiting is an important factor for monitoring bioaccumulation of trace elements both in the fish tissues, and in the intestinal parasites. Younger fish infested with nematodes tended to have higher levels of trace elements. The proportion of all trace elements in parasites collected from younger fish was higher than that in the parasites of older fish and from all the host tissues tested. Therefore, the age of fish sampled needs to be recorded and considered while evaluating trace element pollutions. Moreover, intestinal parasites of whiting shoved high difference in terms of trace element levels comparing to fish tissues, and endoparasites could be therefore a more appropriate tool for determining trace element dynamics and pollution in an ecosystem.

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REFERENCES

[1] Adriano, D.C. (2001): Trace Elements in Terrestrial Environments: Biogeochemistry, Bioavailability and Risks of Metals - Sheridan Books Inc., Ann Arbor, MI.

[2] Altuntas, C., Ogut, H. (2010): Monthly occurrence and prevalence of viral haemorrhagic septicaemia virus (VHSV) in whiting Merlangius merlangus. - Diseases of Aquatic Organisms 88: 107-113.

[3] Azmat, R., Fayyaz, S., Kazi, N., Mahmood, S.J., Uddin, F. (2008): Natural Bioremediation of Heavy Metals Through Nematode Parasite of Fish. - Biotechnology 7: 139-143.

[4] Boyce, N.P., Yamada, S.B. (1977): Effects of a parasite, Eubothrium salvelini (Cestoda: Pseudophyllidea), on the resistance of juvenile sockeye salmon Oncorhynchus nerka to zinc. - Journal of the Fisheries Research Board of Canada 34: 706-709.

[5] Ciloglu, E., Sahin, C., Zengin, M., Genc, Y. (2001): Determination of some population parameters and reproduction period of whiting (Merlangius merlangus euxinus Nordmann, 1840) on the Trabzon-Yomra coast in the eastern Black Sea. - Turkish Journal of Veterinary and Animal Sciences. 25: 831-837.

[6] Dural, M., Genc, E., Sangun, K., Guner, O. (2011): Accumulation of some heavy metals in Hysterothylacium aduncum (Nematoda) and its host sea bream, Sparus aurata

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DOI: 10.15666/aeer/1303_741752

(Sparidae) from North-Eastern Mediterranean Sea (Iskenderun Bay). - Environmental Monitoring and Assessment 174: 147-155.

[7] Eira, C., Torres, J., Miquel, J., Vaqueiro, J., Soares, A.M.V.M., Vingada, J. (2009): Trace element concentrations in Proteocephalus macrocephalus (Cestoda) and Anguillicola crassus (Nematoda) in comparison to their fish host, Anguilla anguilla in Ria de Aveiro, Portugal. - Science of The Total Environment 407: 991–998.

[8] Gabrashanska, M., Nedeva, I. (1996): Content of heavy metals in the sistem flsh-cestodes, VII European Multicolloquium of Parasitology, Parma. Italy.

[9] Galli, P., Crosa, G., Ambrogi, A.O. (1998): Heavy metals concentrations in acanthocephalans parasites compared to their fish host. - Chemosphere 37: 2983-2988.

[10] Gunkel, G. (1994): Bioindikation in Aquatischen ökosystemen Verlag, Stuttgart, Germany.

[11] Morsy, K., Bashtar, A.R., Abdel-Ghaffar, F., Mehlhorn, H., Quraishy, S.A., El-Mahdi, M., Al-Ghamdi, A., Mostafa, N. (2012): First record of anisakid juveniles (Nematoda) in the European seabass Dicentrarchus labrax (family: Moronidae), and their role as bio-indicators of heavy metal pollution. - Parasitology Research 110: 1131-8.

[12] Pascoe, D., Cram, P. (1977): The effect of parasitism on the toxicity of cadmium to the three-spined stickleback, Gasterosteus aculeatus L. - Journal of Fish Biology 10: 467-472.

[13] Retief, N.R., Avenant-Oldewage, A., Du Preez, H.H. (2009): Seasonal study on Bothriocephalus as indicator of metal pollution in yellowfish, - South Africa. Water SA 35: 315-322.

[14] Sims, D. (1996): The effect of body size on the standard metabolic rate of the lesser spotted dogfish. - Journal of Fish Biology 48: 542-544.

[15] Skryabin, K.I. (1969): Key to parasitic nematodes. Vol. I. Spirurata and Filariata. -Israel Program for Scientific Translations.

[16] Sures, B. (2003): Accumulation of heavy metals by intestinal helminths in fish: an overview and perspective. - Parasitology 126 Suppl: S53-60.

[17] Sures, B., Siddall, R. (1999): Pomphorhynchus laevis: the intestinal acanthocephalan as a lead sink for its fish host, chub (Leuciscus cephalus). - Experimental Parasitology 93: 66-72.

[18] Sures, B., Taraschewski, H., Jackwerth, E. (1994): Lead content of Paratenuisentis ambiguus (Acanthocephala), Anguillicola crassus (Nematodes) and their host Anguilla anguilla. - Diseases of Aquatic Organisms 19: 105-107.

[19] Sures, B., Siddall, R., Taraschewski, H. (1999a): Parasites as accumulation indicators of heavy metal pollution. - Parasitology Today 15: 16-21.

[20] Sures, B., Steiner, W., Rydlo, M., Taraschewski, H. (1999b): Concentrations of 17 elements in the zebra mussel (Dreissena polymorpha), in different tissues of perch (Perca fluviatilis), and in perch intestinal parasites (Acanthocephalus lucii) from the subalpine lake Mondsee, Austria. - Environmental Toxicology and Chemistry 18: 2574-2579.

[21] Szefer, P., Rokicki, J., Frelek, K., Skora, K., Malinga, M. (1998): Bioaccumulation of selected trace elements in lung nematodes, Pseudalius inflexus, of harbor porpoise (Phocoena phocoena) in a Polish zone of the Baltic Sea. - Science of the Total Environment 220: 19-24.

[22] Vinodhini, R., Narayanan, M. (2008): Bioaccumulation of heavy metals in organs of fresh water fish Cyprinus carpio (Common carp). - International Journal of Environmental Science and Technology 5: 179-182.

[23] Zimmermann, S., Baumann, U., Taraschewski, H., Sures, B. (2004): Accumulation and distribution of platinum and rhodium in the European eel Anguilla anguilla following aqueous exposure to metal salts. - Environmental Pollution 127: 195-202.

[24] Zimmermann, S., Menzel, C.M., Stuben, D., Taraschewski, H., Sures, B. (2003): Lipid solubility of the platinum group metals Pt, Pd and Rh in dependence on the presence of complexing agents. - Environmental Pollution 124: 1-5.

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