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Accumulation of some heavy metals in *Hysterothylacium aduncum* (Nematoda) and its host sea bream, *Sparus aurata* (Sparidae) from North-Eastern Mediterranean Sea (Iskenderun Bay)

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Abstract The sea bream's nematode and *Sparus aurata*, sampled from the Iskenderun Bay, Mediterranean, in March 2008 were analyzed by inductively coupled plasma–atomic emission spectrometry for their some heavy metal (Cd, Cr, Cu, Fe, Hg, Mn, Mg, Pb, and Zn) levels. The metal concentrations of the parasites were compared to different organs (liver, muscle, gill, intestine, and skin) of the fish hosts. There were significant differences in Cd, Cr, Cu, Fe, Mn, Zn, Hg, Mg, and Pb concentrations in tissues of fish and its parasite. The parasite Cd, Cu, and Pb concentration was higher than the other tissues. Furthermore, significant differences were detected in the heavy metal accumulations between the parasitized and unparasitized fish tissues in Cd, Cu, Hg, and Pb concentrations. The Cd, Hg, and Pb concentrations were found in fish muscle at mean concentrations over the permissible limits proposed by the Food and Agriculture Organization.

Keywords Anisakidae · Fish · Parasite–host system · Metals · Pollution monitoring

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Introduction

A variety of organisms have been investigated to evaluate their potential as biological indicators of different forms of pollution in the aquatic environment. The relationship between pollution and parasitism in aquatic organisms and the potential role of parasites as water quality indicators have received increasing attention during the past two decades. However, until recently, little was known about the accumulation of toxins within parasites. Certain parasites, particularly intestinal helminthes of fish, can accumulate heavy metals at concentrations that are orders of magnitude higher than those in the host tissues or the environment (Sures et al. 1999). A variety of wild freshwater and marine fish are subject to infection by different species of parasites. This aspect in addition to their capacity to accumulate heavy metals suggest that parasites may serve as useful indicators for biologically available metals in aquatic ecosystems that current methods of water

and sediment analyses cannot accurately measure (Galli et al. 1998).

Among many parasites found in fishes, nematodes are those of the greatest importance, their larvae living both in viscera and tissues of marine fishes. Some species bring about serious zoonoses in humans, in most cases a surgical treatment being required. The *Anisakis* simplex larvae belong to this group of nematodes. The first identified *Anisakis*-caused case was noted in 1955 in Holland, and from then on, the anisakidosis was recorded both in Holland and other European countries; the population of Japan, habitually consuming raw fish, is particularly vulnerable to the disease (Grabda 1976).

Kennedy (1997) suggested that if the use of parasites as indicators of pollution can be justified, the fish host species must be abundant and easily accessible, and the species of parasite, despite their overdispersed distribution, must show a high prevalence and abundance in the fish population. Parasites should also be easily removed and counted, and details of the ecology and biology of both fish host and parasites should be available. Schludermann et al. (2003) claimed that some trace toxic metals have been found to higher extent in parasite than in tissue of their host organism and to demonstrate a good suitability as a biomonitor of environmental pollution (Azmat et al. 2008).

Nematodes of the family Anisakidae parasitize fish, mammals, birds, and reptiles, with the larval stages of some species causing severe clinical disease in humans. Anisakidae characteristically occur in deep waters in meso- or benthopelagic species, and they are typically found in predators. Natural transmission also occurs in specific habitats and in relation to characteristic host diets (Abollo et al. 2001). Anisakid nematodes of the genus *Hysterothylacium* use fish as both intermediate and definitive hosts, in which they attain maturity.

Anisakid parasites, *Hysterothylacium* sp., was found in sparid fishes with 1.74% prevalence level (Genc 2002) and also elasmobranch fishes 7.69–78.57% prevalence levels in North-eastern Mediterranean Sea, Iskenderun Bay (Genc et al. 2005). Previous researches pointed out *Hysterothylacium* infection all around the world including the Mediterranean Sea (Petter and Cabaret 1995; Petter and Maillard 1988; Costa et al. 2004; Anderson 2000; Henderson et al. 2002; Marques et al. 2005; Valero et al. 2006).

It has been shown that significantly higher quantity of heavy metals was concentrated in tissues of endoparasites than in their fish host (Sures 2003, 2004). For example, mean concentrations of lead and cadmium in *Monobothrium wageneri* (cestode) from intestine of tench (*Tinca tinca*) were, respectively, 75 and 40 times higher than

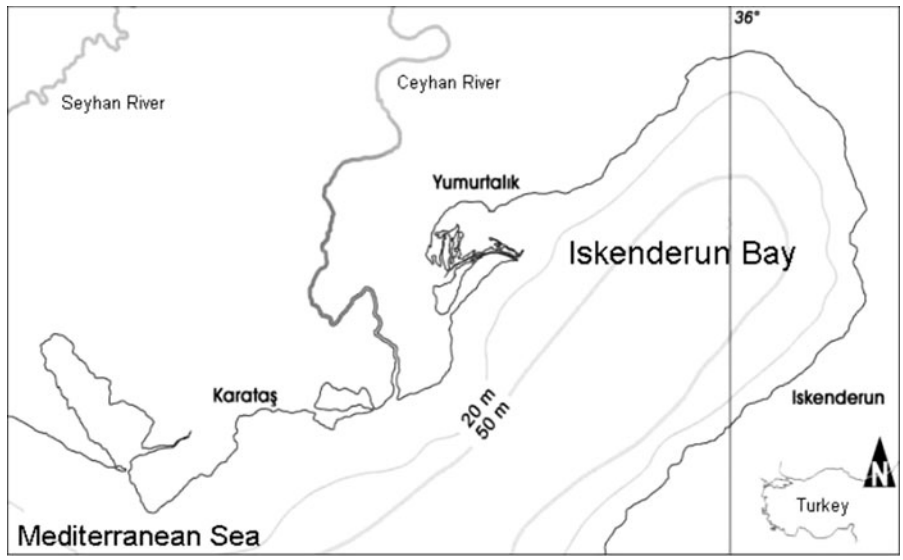

Fig. 1 The map of sampling locations

in the muscle of the host (Sures et al. 1997). In addition, Tenora et al. (2000) investigated Cr, Pb, and Cd concentrations in the *Ligula intestinalis* plerocercoids (cestode) and three of its intermediate hosts. Helminthes show a high capacity of bioaccumulation of heavy metals located in the intestine of their respective final host.

Iskenderun Bay is situated on the North Eastern Mediterranean coast of Turkey (36°20′ N–35°30′ E; 36°50′ N–35°00′ E; Fig. 1). This area, in which there are large quantities of midtreated industrial and domestic sewages, has one of the polluted coastal waters of Turkey. This bay has also an economic importance for fishery. The sea bream, *Sparus aurata* (Sparidae: Teleostei), is a fish species with high commercial value, in Mediterranean countries. Heavy metal concentrations in several fish species from the Iskenderun Bay of the Mediterranean Sea were determined by Yılmaz (2003). The range of metal concentrations in muscle were as follows: Fe, 41.84–70.28; Ni, 0.94–1.22; Cr, 1.28–1.46; Pb, 1.03–7.45; and Zn, 19.55–38.23 (wet wt). In addition, heavy metal concentrations in *S. aurata* from Iskenderun Bay were determined by Yılmaz (2005). The concentrations were as follows: Fe, 20.65–28.81; Cu,0.32–0.51; Ni,0.29–0.87; Cr, 0.86–1.08; Pb, 4.84–7.33; and Zn, 19.31–31.23.

The above-mentioned examples demonstrate the great importance and overall presence of parasites in aquatic ecosystems. Due to their wide abundance and distribution, several researchers started to focus on the use of parasites as indicators of environmental quality. Our specific objective was to determine whether the valid *Anisakids* are a useful bioindicator species as part of a parasite–host system with sea bream, *S. aurata*, which is highly likely to be exposed to waterborne contaminants.

Materials and methods

Forty-six *S. aurata* were collected, using spherical fish pots (50 × 45 cm) in Iskenderun Bay on March 2008. The samples were brought to the laboratory (in a 500-L plastic aerated tank) on the same day immediately, and all fish were measured by over anaesthetization in an aerated

Table 1 Total length-weight and intensity of *H. aduncum* in Sea bream (*S. aurata*)

Sample	*n*	TL±SD (range, cm)	W±SD (range, g)	The status of infection			
				P (%)	MI±SD	Total number of parasites (range)	TL_p (range, mm)
Parasitized	25	17.67 ± 2.05 (13.80–22.40)	139.34 ± 25.52 (87.30–173.32)	45.65	6.29 ± 3.10	132 (2–13)	39.3–88.9
Unparasitized	21	18.07 ± 3.27 (18.98–38.12)	145.07 ± 32.83 (92.80–192.10)	ND	ND	ND	ND

W mean weight of fish, *TL* mean total length of fish, *TLp* the ranges of total lengths of parasites, *P*(%) prevalence, *MI* mean intensity, *SD* standard deviation, *ND* not detected

water bath containing 10-mg/L quinaldine sulfate (Sigma Chemical Company, Germany; Yanar and Genc 2004; Genc et al. 2008). The live weight (grams), lengths (centimeters), sex, and age of fish samples are given in Table 1. The nematodes were collected separately from each intestine of different fish. Approximately 4 g of the muscle on the surface of the fish (epaxial muscle of dorsal side), entire liver, intestine, gill, and skin (ventrolateral side) from each fish were dissected, washed with distilled water, packed in polyethylene bags, and kept at −21°C until analysis.

Sample preparation and analysis were carried out according to the procedure described by UNEP/FAO/IOC/IAEA (1984). Tissues digested with concentrated nitric acid and percholoric acid (2:1 v/v) at 60°C for 3 days and for mercury analysis samples were digested using HNO_3 for the determination of mercury in closed system. All samples were diluted with bidistilled water and assayed using inductively coupled plasma–atomic emission spectrometry (Varian model—Liberty Series II). All digested samples were analyzed three times for each metals. The standard addition method was used to correct for matrix effects. Standards were prepared from stock standard solution of metals. All chemicals and standard solutions used in the study were obtained from Merck and were of analytical grade.

Intercalibration homogenate samples (International Atomic Energy Agency) were used as a quality control for the analytical methodology. The accuracy of analytical procedure was checked by analyzing the standard reference materials (National Research Council of Canada; dogfish muscle and liver MA-A-2/TM Fish Flesh) DORM-2 in two replicates for each batch of 46 samples digested. Recovery rates ranged from 96% to 106% for all investigated elements (Table 2). The absorption wavelength and detection limits are shown in Table 3.

Table 3 The absorbtion wavelengths and limits of detection

Element	Wavelength (nm)	Detection limits
Cd	228.804	0.001
Cu	324.750	0.010
Fe	238.200	0.0046
Mn	257.610	0.0014
Pb	220.350	0.0042
Zn	206.200	0.0059
Hg	193.696	0.0002
Cr	283.600	0.001
Mn	257.610	0.001

The metal concentration (Cd, Cr, Cu, Fe, Hg, Mn, Mg, Pb, and Zn) in tissue was recorded as microgram metal per gram wet weight. Prior to the analyses, all data were checked for outliers, and homogeneity of variance was also tested. Differences between parasitized and unparasitized fish tissues for any metal concentration were checked by "Data analyses" built in MS Excel. Analysis of variance (ANOVA) was used to evaluate the effect of parasite hosting over the metal accumulation in tissues. Then, Duncan multiple range test was performed if significant difference was found in ANOVA. To compensate for the increased likelihood of type I error, a setting of $\alpha = 0.05$ was used. The data were examined for homogeneity of variance, and Scheffe's post hoc test was used for equal variance conditions, and Dunnett's T3 post hoc test was used for unequal variance conditions following the ANOVA.

Table 2 Observed and certified values of elemental concentrations as micrograms per gram dry weight in standard reference materials DORM-2 from the Nationals Research Council, Canada ($n = 2$)

DORM-2	Certified values (μg/g)	Measured values	Recovery (%)
Zn	25.6 ± 2.3	24.9 ± 2.4	97
Fe	142 ± 10	137 ± 11	96
Cd	0.043 ± 0.008	0.045 ± 0.009	104
Cu	2.34 ± 0.16	2.26 ± 0.17	96
Pb	0.065 ± 0.007	0.069 ± 0.008	106
Cr	0.200 ± 0.01	0.199 ± 0.009	99
Mn	0.050 ± 0.006	0.0485 ± 0.007	97
Hg	0.050 ± 0.007	0.0489 ± 0.008	97

Table 4 Mean metal concentrations (milligrams per kilogram wet weight) in tissues of unparasitized *S. aurata* (mean value ± SE of repeating measurement)

	Skin	Muscle	Gill	Liver	Intestine
Cd	2.044 ± 0.957^{a}	1.255 ± 0.793^{ab}	0.188 ± 0.004^{b}	0.742 ± 0.129^{ab}	0.433 ± 0.006^{ab}
Cr	1.534 ± 0.839^{a}	0.573 ± 0.281^{a}	3.920 ± 0.424^{b}	0.456 ± 0.210^{a}	0.456 ± 0.143^{a}
Cu	19.583 ± 7.512^{a}	6.237 ± 1.450^{b}	8.264 ± 1.757^{ab}	18.870 ± 2.957^{ab}	16.905 ± 4.118^{ab}
Fe	63.194 ± 7.378^{ab}	17.310 ± 3.472^{a}	33.349 ± 4.557^{ab}	357.217 ± 69.461^{b}	124.743 ± 16.850^{c}
Hg	1.380 ± 0.601^{a}	7.845 ± 5.771^{b}	4.490 ± 3.072^{c}	1.282 ± 0.680^{a}	0.816 ± 0.523^{a}
Mg	1064.5 ± 172.17^{ab}	762.89 ± 98.27^{a}	1875.1 ± 276.0^{c}	658.82 ± 64.43^{a}	1278.6 ± 132.49^{b}
Mn	3.946 ± 0.902^{a}	2.006 ± 0.621^{a}	20.433 ± 0.041^{c}	4.115 ± 0.933^{a}	12.728 ± 6.346^{b}
Pb	3.052 ± 0.594	3.830 ± 1.445	2.428 ± 0.494	2.658 ± 0.614	2.032 ± 0.477
Zn	133.38 ± 19.951^{c}	14.349 ± 2.427^{a}	141.807 ± 15.236^{c}	58.746 ± 6.463^{b}	38.165 ± 3.767^{ab}

*Mean values in lines with different superscript (a to c) are significantly different ($P < 0.05$)

Results

In this study, 25 out of 46 specimens were found infected by *Hysterothylacium aduncum* (Rudolphi 1802) collected from the Iskenderun Bay in 2008. The total number of parasites (range), prevalence (%), and mean intensity values of samples were 132 (2–13), 45.65%, and 6.29 ± 3.10, respectively.

The concentrations of heavy metals in unparasitized fish tissues (gill, liver, muscle, intestine, and skin) are given in Table 4. Although there were no significant differences between Pb concentrations in tissues of unparasitized *S. aurata*, especially the Cr, Mg, Mn, and Zn concentrations in the gill, Cu and Cd concentrations in the skin, and Fe concentrations in the liver are higher than those in the muscle tissues ($P < 0.05$).

The concentrations of heavy metals in fish tissues and its parasite *H. aduncum* are given in Table 5. Mean concentrations of Cd, Cu, and Pb in parasite were higher than those in the other tissues of host fish. Although there were significant differences in all metal concentrations in tissues of fish and its parasite, Cr, Mg, Mn, and Zn concentrations in the gill, Fe concentrations in the liver, and Hg concentrations in the intestine are higher than those in the muscle tissues. Furthermore, significant differences were detected in the heavy metal accumulations between the parasitized and unparasitized fish tissues in Cd, Cu, Hg, and Pb concentrations.

Discussion

Certain organisms are able to provide information about the chemical state of their environment through their presence or absence. Others are less affected by toxic substances but show an ability to concentrate environmental pollutants inside their tissues (Rosenberg and Resh 1993). Thus far, studies have mainly focused on

Table 5 Mean metal concentrations (milligrams per kilogram wet weight) in tissues of *S. aurata* and in the parasite *H. aduncum* (mean value ± SE of repeating measurement)

	Skin	Muscle	Gill	Liver	Intestine	Parasite
Cd	0.281 ± 0.009^{a}	0.427 ± 0.007^{ab}	0.166 ± 0.003^{a}	0.656 ± 0.009^{ab}	0.620 ± 0.107^{ab}	2.048 ± 1.308^{b}
Cr	0.331 ± 0.009^{a}	0.125 ± 0.005^{a}	4.509 ± 0.294^{b}	0.115 ± 0.007^{a}	0.167 ± 0.008^{a}	1.390 ± 0.935^{a}
Cu	7.525 ± 0.662^{a}	5.892 ± 1.112^{a}	14.305 ± 5.264^{ab}	17.153 ± 5.148^{ab}	13.976 ± 2.420^{ab}	28.012 ± 9.454^{b}
Fe	48.998 ± 11.51^{a}	34.187 ± 14.41^{a}	53.765 ± 14.78^{a}	323.91 ± 99.27^{b}	199.87 ± 78.27^{ab}	102.26 ± 54.82^{a}
Hg	1.041 ± 0.656	1.509 ± 0.725	0.959 ± 0.522	1.393 ± 0.639	2.301 ± 0.364	1.275 ± 0.457
Mg	905.80 ± 162.45^{a}	720.51 ± 65.11^{a}	2187.13 ± 96.51^{b}	665.55 ± 148.73^{a}	923.38 ± 253.33^{a}	971.21 ± 412.71^{a}
Mn	2.396 ± 0.773^{a}	1.434 ± 0.892^{a}	14.392 ± 2.245^{b}	3.289 ± 0.649^{a}	4.797 ± 1.531^{a}	3.598 ± 1.726^{a}
Pb	1.801 ± 0.289	4.459 ± 1.712	3.818 ± 0.798	1.756 ± 0.426	3.214 ± 0.842	7.289 ± 4.419
Zn	96.730 ± 16.51^{c}	13.531 ± 1.555^{a}	141.32 ± 7.654^{d}	50.568 ± 9.617^{ab}	41.920 ± 6.733^{ab}	62.554 ± 26.810^{bc}

*Mean values in lines with different superscript (a to d) are significantly different ($P < 0.05$)

the use of parasites as accumulation indicators of heavy metals; less frequently, investigations have dealt with organic pollutants. This is obviously related to different accumulation patterns of hydrophilic and lipophilic substances. Lipophilic chemicals mainly accumulate in fat and therefore become biomagnified along foodwebs, whereas hydrophilic substances are distributed more evenly among tissues. Parasites, having a low percentage of fat, are not able to bioconcentrate lipophilic substances above the levels of the host tissues (Heinonen et al. 1999). Even if parasites do not accumulate organic pollutants, they are able to alter the uptake of chemicals of their hosts, including metals (Evans et al. 2001).

Knowledge of fish parasites is of particular interest in relation not only to fish health but also to understanding of ecological problems. It is also necessary from a public health viewpoint to determine the heavy metal concentrations in fish captured for human consumption, and the analysis of parasites from their hosts could provide a sensitive indirect measure of this (Sures 2004). Although the majority of parasitological papers have dealt with parasites as a threat for the health of fish (Schäperclaus 1990), several hundred papers have been published since 1980 that are directly concerned with the relationship between pollution and parasitism in the aquatic environment (Khan and Thulin 1991; Poulin 1992; Vethaak and Rheinallt 1992; Overstreet 1993; MacKenzie et al. 1995; Lafferty 1997; Kennedy et al. 1998; Sures et al. 1997, 1999). This increasing interest especially in fish parasites is related with the high number of parasite species commonly found in or on freshwater and marine fish.

In the North Atlantic, the North Sea, the Baltic Sea, the Mediterranean Sea, and adjacent temperate and cold waters, the species *H. aduncum* is a very common fish parasite (Palm et al. 1999). In addition, Genc (2002) reported that *Hysterothylacium* sp. was found in sparid fish (Sparidae) with a 1.74% prevalence level from Eastern Mediterranean of Turkey.

Anisakid nematodes of the genus *Hysterothylacium* use fish as both intermediate and definitive hosts, in which they attain maturity. Some *Hysterothylacium* species were reported from sparid fishes, namely, *Pagellus acarne* (Petter and Cabaret 1995) and a related species *Diplodus sargus* from the Mediterranean (Petter and Maillard 1988).

Azmat et al. (2008) worked on the bioaccumulation potential of heavy toxic metals, which was assessed in *Echinocephalus* sp. and *Ascaris* sp., which were reported as natural bioremediators of heavy metals in *Liza vaigiensis* from Karachi coast. Investigation suggests that infected fish contain low concentration of heavy metals in their muscle as compared to noninfected one. The high level of toxic metals in *Echinocephalus* sp. and *Ascaris* sp. within its host suggests that these nematode parasites are sensitive indicator of heavy metals in aquatic ecosystem showing sharing of more burden of environmental pollution of sea and also act as bioremediator of heavy metals in fish. In the present study, metal concentrations of the parasites were compared to those of the different organs (intestine, gill, liver, muscle, and skin) of the fish hosts. Bioconcentrations of Cd, Cr, Fe, Cu, Hg, Mg, Mn, Pb, and Zn were detected in the *H. aduncum*, and generally, it contained statistical differences with the other tissues of its host, the sea bream ($P > 0.05$).

The highest concentrations in parasites appear to be found in parasites that live within the host's intestines. Acanthocephalans and tapeworms in the intestines of fish appear to have the greatest ability to concentrate metals (Sures et al. 1995, 1997). Lower accumulations were seen in cestode larvae in the body cavity of fish intermediate hosts (Gabrishanska and Nedeva 1996; Pascoe and Cram 1977). In contrast, nematode parasites, *Anguillicola crassus*, accumulated less Pb than their host, the eel *Anguilla anguilla* (Sures et al. 1994). However, other studies of nematodes (Tenora et al. 1999, 2000) indicate greater concentrations of metals in the nematode than in the fish host. The developmental stage of the parasite and the amount of time the parasite is living in a particular host are other factors that can influence metal accumulation. Differences in relative bioaccumulation can, therefore, be due to characteristics of the particular parasite, its developmental stage, the metal, the parasite's location in the host, and the host. While *Eustrongylides* sp. accumulated about two thirds of the Hg level of their fish host's muscle, *Probopyrus* accumulated

less than half of the Hg concentration of their host *Probopyrus pugio*. Neither of these parasites live within the intestines of their host, but these parasites get nutrition from hemolymph and other body fluids. This reduced uptake in the parasites occurred despite the fact that methylmercury does biomagnify (Suedel et al. 1994).

Sures (2004) summarized and documented serious arguments to establish selected parasites (helminthes–acanthocephalans and tapeworms) as sentinels, with comments that nematodes of mammals, similar to nematodes of fish, do not appear to be very efficient as accumulation indicators owing to their low metal levels.

The results presented in this study provide support for further investigations into these common organisms and their bioindicating properties. From the data presented here, one may conclude that the intestine nematode *H. aduncum* accumulates more Cd, Cu, and Pb than the tissues of its final host sea bream. Furthermore, Cd, Hg, and Pb concentrations were found in fish muscle at mean concentrations over the permissible limits proposed by FAO (1983). Recent field studies have demonstrated that particular fish parasites can accumulate toxic metals from the aquatic environment. Thus, the application of certain parasites as sentinel organisms could provide a promising new domain for future research in environmental parasitology research.

It is clear that we are still lacking considerable amounts of information on the effects of parasites on common bioindication procedures such as the analysis of biomarkers and the use of parasites as indicators. We need much more knowledge about the basic physiological effects that parasites have on their hosts at the time of infection, development, and reproduction. If we understand these basic processes, ecotoxicological research could benefit from this (e.g., with respect to evaluating the meaningfulness of biomarker reaction in relation to pollution). This is a field of growing importance, where parasitologists should try to combine their knowledge and expertise with toxicologists and ecotoxicologists. Furthermore, it appears that parasites themselves might be valuable as effect indicators, if changes of populations or communities are monitored. This is especially advantageous to indicate sustainable long-term changes of ecosystems. Yet, again, more information is required to establish certain host–parasite assemblages as effect indication systems. Finally, the remaining need for sentinels in terrestrial habitats should encourage scientists to intensify research in this interdisciplinary area.

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