Journal of Helminthology

cambridge.org/jhl

Trace element assessment in *Neoechinorhynchus agilis* (Rudolphi, 1918) (Acanthocephala: Neoechinorhynchidae) and its fish hosts, *Mugil cephalus* (Linnaeus, 1758) and *Chelon ramada* (Risso, 1827) from Ichkeul Lagoon, Tunisia

H. Jmii Chine[1], M. Nachev[2], B. Sures[2] and L. Gargouri[1]

[1]Faculty of Sciences of Tunis, Laboratory of Diversity, Management and Conservation of Biological Systems, University of Tunis El Manar, LR18ES06, Tunis, Tunisia and [2]Department of Aquatic Ecology and Centre for Water and Environmental Research, University of Duisburg-Essen, Universitätsstr. 5, 45141, Essen, Germany

Research Paper

Cite this article: Jmii Chine H, Nachev M, Sures B, Gargouri L (2021). Trace element assessment in *Neoechinorhynchus agilis* (Rudolphi, 1918) (Acanthocephala: Neoechinorhynchidae) and its fish hosts, *Mugil cephalus* (Linnaeus, 1758) and *Chelon ramada* (Risso, 1827) from Ichkeul Lagoon, Tunisia. *Journal of Helminthology* **95**, e61, 1–7. https://doi.org/10.1017/S0022149X21000572

Received: 4 August 2021
Revised: 3 October 2021
Accepted: 4 October 2021

Keywords:
Neoechinorhynchus agilis; metal uptake; mullets; pollution; brackish water

Author for correspondence:
H. Jmii Chine,
E-mail: halimajmii@hotmail.com

Abstract

Acanthocephalans belonging to the species *Neoechinorhynchus agilis* were collected from two mullets, *Mugil cephalus* and *Chelon ramada* from Ichkeul Lagoon in northern Tunisia. Collected parasites, as well as tissues of their hosts (muscle, liver and intestine), were analysed for trace elements (silver, arsenic, cadmium, cobalt, copper, iron, manganese, nickel (Ni), lead (Pb), selenium, vanadium (V), zinc) using inductively coupled plasma mass spectrometry. Our results showed different accumulation patterns of trace elements in fish tissues and parasites. Among the host tissues, liver accumulated the highest metal amounts. Acanthocephalans showed Ni, Pb and V in significantly higher concentrations compared to their host's tissues. Further, the calculated bioconcentration factors demonstrated a 390-fold higher Pb accumulation in the parasite compared to fish muscle. This study is the first field survey in Tunisia dealing with elements' uptake in parasites and their hosts. Our results corroborate the usefulness of the acanthocephalans for biomonitoring of metal pollution in aquatic ecosystems and promote more research in order to understand host–parasite systems in brackish waters of the Mediterranean area.

Introduction

Aquatic ecosystems are typically vulnerable to natural and anthropogenic stressors (Birk *et al.*, 2020). Among the main stressors, various elements (mainly heavy metals) can become highly toxic for biota as soon as their concentrations exceed natural values (Merian *et al.*, 2004). Due to their uptake by biota and potential toxic effects on it, studies using living organisms as bioindicators for metals pollution have shown a great interest in recent decades. Bioindicators are helpful for quantification of bioavailable fraction of pollutants and for elucidating the uptake and storage mechanisms of pollutants in organisms (Hamza-Chaffai, 2014). Many strategies have been developed to monitor and evaluate the impact of metals in aquatic ecosystems and various organisms, animals in particular, have been used in monitoring programs. Molluscs, especially bivalves, are well known for their ability to accumulate huge amounts of metals and other kinds of pollutants (Rosenberg & Resh, 1993; Sures *et al.*, 1997; Kefi *et al.*, 2016). Their feeding behaviour as active filter feeders and their tolerance to a large range of environmental conditions made bivalves good candidates for aquatic pollution biomonitoring.

Recently, in addition to the previously mentioned free-living sentinel species, parasites and host–parasite systems were found to be sensitive bioindicators for aquatic pollution. Among different helminths, acanthocephalans of fish have been promoted as sensitive sentinels for metal pollution in aquatic environments (summarized in Sures *et al.*, 2017). Acanthocephalans can accumulate trace elements thousands times higher than their fish hosts and their ambient environment (Sures, 2004, 2005; Nachev *et al.*, 2010) and, therefore, they can be even more efficient in environmental monitoring than free-living sentinel organisms such as mussels (Sures *et al.*, 1997). According to Sures (2003), they constitute ideal sentinels and a sensitive tool for monitoring metal availability in aquatic ecosystems. However, the majority of studies focused on freshwater ecosystems, while marine ecosystems and specifically lagoons have rarely been considered (Nachev & Sures, 2015). Lagoons with their differing environmental conditions can harbour many different species of fish, including mugilids (Bone & Moore, 2008). Mugilids are known to be hosts for acanthocephalans belonging to the genus *Neoechinorhynchus*, such as the species *Neoechinorhynchus agilis* (Jithendran & Kannappan 2010; Tkach *et al*., 2014).

The aim of the present study was to assess the element concentrations in two mugilid fish species (*Mugil cephalus* and *Chelon ramada*) from brackish water habitats and in their acanthocephalan parasite *N. agilis*. In addition, we tried to evaluate the potential risk to human health since those two fish species are frequently taken for human consumption. This field survey was performed in one of the most important coastal lagoons in Tunisia (Ichkeul Lagoon), which is known to be impacted by combined pollution from natural and anthropogenic sources (Ben Mbarek, 2001; Ouchir *et al*., 2016). Both the water and sediments in this lagoon contain high concentrations of heavy metals such as copper (Cu), iron (Fe), lead (Pb) and zinc (Zn) (Ouchir *et al*., 2016; Yazidi *et al*., 2017).

Material and methods

Sample collection

Two different mullet species, *M. cephalus* and *C. ramada*, were caught from Ichkeul Lagoon, northern Tunisia, by local fishermen in the period from March 2016 to September 2017. After collection, they were transported immediately to the laboratory and examined for acanthocephalans. Tissue samples (muscle, liver, intestine) were taken carefully with the aid of stainless steel scissors and forceps. The acanthocephalans were removed from the fish intestine and the remaining intestinal tissue was washed with distilled water to avoid any remains of worms and gut content. All samples and parasites were stored at −20°C until further analyses.

Analytical procedure

Samples of fish tissues and parasite were digested using the microwave digestion system Mars 6 equipped with 20 ml MARSXpress PFA vessels (both CEM Corporation, Kamp Lintfort, Germany). The procedure consists of placing a previously homogenized amount of the sample (up to 250 mg of wet weight) into vessels, into which 4 ml of nitric acid (65% HNO_3, sub boiled) was added. The vessels were heated for 90 min at 170°C using the microwave digestive system. Subsequently, the clear sample solution was brought to 5 ml volume with deionized water (Merck Millipore, Burlington, USA) in a volumetric glass flask. The elements, silver (Ag), arsenic (As), cadmium (Cd), cobalt (Co), Cu, Fe, manganese (Mn), nickel (Ni), Pb, selenium (Se), vanadium (V) and Zn have been quantified in mullet's tissue and their parasite *N. agilis* using inductively coupled plasma mass spectrometry (ICP-MS) (Elan 6000, Perkin Elmer, Waltham, USA; for details on instrument settings and calibration, see Nachev *et al*., 2010). The analytical procedure was validated using standard reference materials (1566b Oyster tissue, National Institute of Standards and Technology, USA; DORM 3, National Research Council, Canada) and detection limits for the investigated elements were calculated as the three-fold standard deviation of concentrations determined in ten procedural blanks.

Data analysis and statistical treatments

Bioconcentration factors (BCF) were calculated according to Sures *et al*. (1999) as follows: $\mathrm{BCF} = (C_{[N.\ agilis]} / C_{[\mathrm{host\ tissue}]})$. The detection limit was used to calculate the BCF in the case when the tissue concentrations were below the detection limits. In order to compare the element concentrations in fish tissues parasites, a Wilcoxon matched pair's test was applied. All statistical analyses were carried out using SPSS statistical package software version 20.0 (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp. IBM Corp. Released 2012).

Results

Analytical procedure

Mean concentrations of the analysed elements in the standard reference materials and the detection limits are listed in table 1. The recovery rates of all 12 elements were within the 20% deviation range.

Element concentrations in mullets and their acanthocephalan

The metal analysis revealed that the concentrations of some elements like Ni, Pb and V were significantly higher in the acanthocephalan than the fish tissues for both mullet species. With the exception of the elements As, Se and V, all other elements were found in significantly higher concentrations in *N. agilis* compared to the muscle and intestine of both fish hosts (tables 2 and 3; figs 1 and 2). Comparisons of element concentrations between fish tissues showed that for both mullets muscle accumulated the lowest amounts of metals, followed by the intestine and then the liver.

According to mean BCFs, five elements (Ag, Mn, Ni, Pb, V) in the *M. cephalus*–*N. agilis* system were overall present in higher levels in the parasites (BCF > 1). Different patterns of metal accumulation capacities were observed for the analysed host tissues. The lowest metal accumulation level was observed in the muscle in comparison to the parasites. The metal accumulation capacity of *N. agilis* with respect to the host muscle was the highest for Cd (up to 495 times higher) followed by Ag (up to 413 times higher) and Pb (up to 390 times higher). The metal accumulation capacity of the parasites with respect to the intestine in decreasing order was as follows: Pb > Ag > Ni > Cd > Cu > Co > V > Zn > Fe > Se > Mn. Regarding the liver, the metal accumulation capacity of the parasites was the highest for the Pb (up to 35 times higher in the acanthocephalan), followed by Ni and V (up to 3.5 times higher in parasites); for the rest of the elements, the mean concentration factors values were lower in parasites (BCF ≤ 1) (table 4).

The calculated BCFs for the *C. ramada*–*N. agilis* system showed an almost similar metal accumulation pattern, but less pronounced than the one observed for the *M. cephalus*–*N. agilis* system. The highest metal accumulation capacity of *N. agilis* with respect to the host muscle was recorded for Ag (up to 823 times higher), followed by Cd (up to 662 times higher), and for the rest of elements the decreasing order of the metal accumulation capacity of the acanthocephalan was as follows: Pb > Ni > Co > Mn > Fe > Zn > Se > As > V. Concerning the intestine and the liver, the highest BCF observed for the parasite was for Pb. The acanthocephalan was able to accumulate this toxic element up to 198 times higher than muscle and up to 40 times higher than liver (table 5).

Discussion

The results of the elements analysis in the two mullet species *M. cephalus* and *C. ramada* showed a difference in the

Table 1. Trace metal concentrations in standard reference material, accuracy and detection limits determined by ICP-MS analyses.

	Oyster tissue 1566b			
Element	Measured values ± SD (µg/g)	Certified values ± SD (µg/g)	Recovery (%)	Detection limit (µg/L)
Ag	0.730 (±0.060)	0.666 (±0.009)	109%	0.042
As	7.09 (±0.41)	7.65 (±0.65)	93%	0.688
Cd	2.51 (±0.20)	2.48 (±0.08)	101%	0.036
Co	0.360 (±0.020)	0.371 (±0.009)	97%	0.028
Cu	69.2 (±3.7)	71.6 (±1.6)	97%	1.420
Fe	163.6 (±19.7)	205.8 (±6.8)	80%	24.683
Mn	18.4 (±1.0)	18.5 (±0.2)	99%	0.149
Ni	0.94 (±0.09)	1.04 (±0.09)	90%	1.321
Pb	0.340 (±0.030)	0.308 (±0.009)	108%	0.113
Se	2.19 (±0.37)	2.06 (±0.15)	106%	5.208
V	0.510 (±0.100)	0.577 (±0.023)	88%	3.458
Zn	1223 (±64)	1424 (±46)	86%	7.750

SD, standard deviation.

Table 2. Differences between element concentrations in *Mugil cephalus* organs and the acanthocephalan *Neoechinorhynchus agilis*.

	P↔M	P↔I	P↔L	M↔I	M↔L	L↔I
Ag	P**	P**	L**	I**	L**	L**
As	NS	NS	L**	I**	L**	L**
Cd	P**	P**	NS	I**	L**	L**
Co	P**	P**	L**	I**	L**	L**
Cu	P**	P**	L**	I**	L**	L**
Fe	P**	P**	L**	I**	L**	L**
Mn	P**	NS	NS	I**	L**	NS
Ni	P**	P**	P**	NS	L**	L**
Pb	P**	P**	P**	I**	L**	L**
Se	NS	NS	L**	I**	L**	L**
V	NS	P**	P**	I**	L**	L**
Zn	P**	P**	L**	I**	L**	L**

M, muscle; I, intestine; L, liver; P, Parasite (in *M. cephalus*).
**Significant at $P \leq 0.01$ (Wilcoxon matched-pair test).
NS, Not significantly different (Wilcoxon matched-pair test).

Table 3. Differences between element concentrations in *Chelon ramada* organs and its acanthocephalan *Neoechinorhynchus agilis*.

	P↔M	P↔I	P↔L	M↔I	M↔L	L↔I
Ag	P**	P**	NS	I**	L**	L**
As	M**	I**	L**	NS	L**	L**
Cd	P**	P**	NS	I**	L**	L**
Co	P**	P**	NS	I**	L**	L**
Cu	P**	P**	NS	I**	L**	L**
Fe	P**	P**	L**	I**	L**	L**
Mn	P**	NS	NS	I**	L**	NS
Ni	P**	P**	P**	NS	L**	L**
Pb	P**	P**	P**	NS	L**	NS
Se	NS	NS	L**	NS	L**	L**
V	P**	P**	P**	I**	L**	L**
Zn	P**	P**	NS	I**	L**	L**

M, muscle; I, intestine; L, liver; P, Parasite (in *C. ramada*).
**Significant at $P \leq 0.01$ (Wilcoxon matched-pair test).
NS, Not significantly different (Wilcoxon matched-pair test).

distribution of the metals' concentrations in host tissues. The liver was the organ that accumulates the highest amount of metals compared to intestine and muscle.

Mullet fish, known by their euryhaline character, have been extensively studied for their ability to accumulate metals (Storelli *et al.*, 2006; Chouba *et al.*, 2007; Fernandes *et al.*, 2007; Annabi *et al.*, 2017; Genç & Yilmaz, 2017). According to Waltham *et al.* (2013), the flathead grey mullet is widely used around the world to monitor pollution in aquatic ecosystems. During our study, the muscle, the intestine and the liver of the fish were examined for metal accumulation. A significant difference was observed in the concentrations of the majority of elements when the tissues of the fish were compared. This variability can be explained by the difference in the physiological role of each organ of the fish (Olsson *et al.*, 1998). Indeed, metals are distributed throughout the cells, but some compartments are particularly important for metal storage. Mechanisms of elements' uptake depend on the nature of the metal and its affinity to the proteins of the cell. Low levels of an element concentration in an organ (like muscle for example) can be attributed to the low level of binding proteins in this organ (Olsson *et al.*, 1998). With respect to the tissues of the two hosts examined in this study, most of the elements were present at the highest levels in the liver and the lowest concentrations were recorded in the muscle. In comparisons to liver, the element concentrations in the intestinal tissue were medium

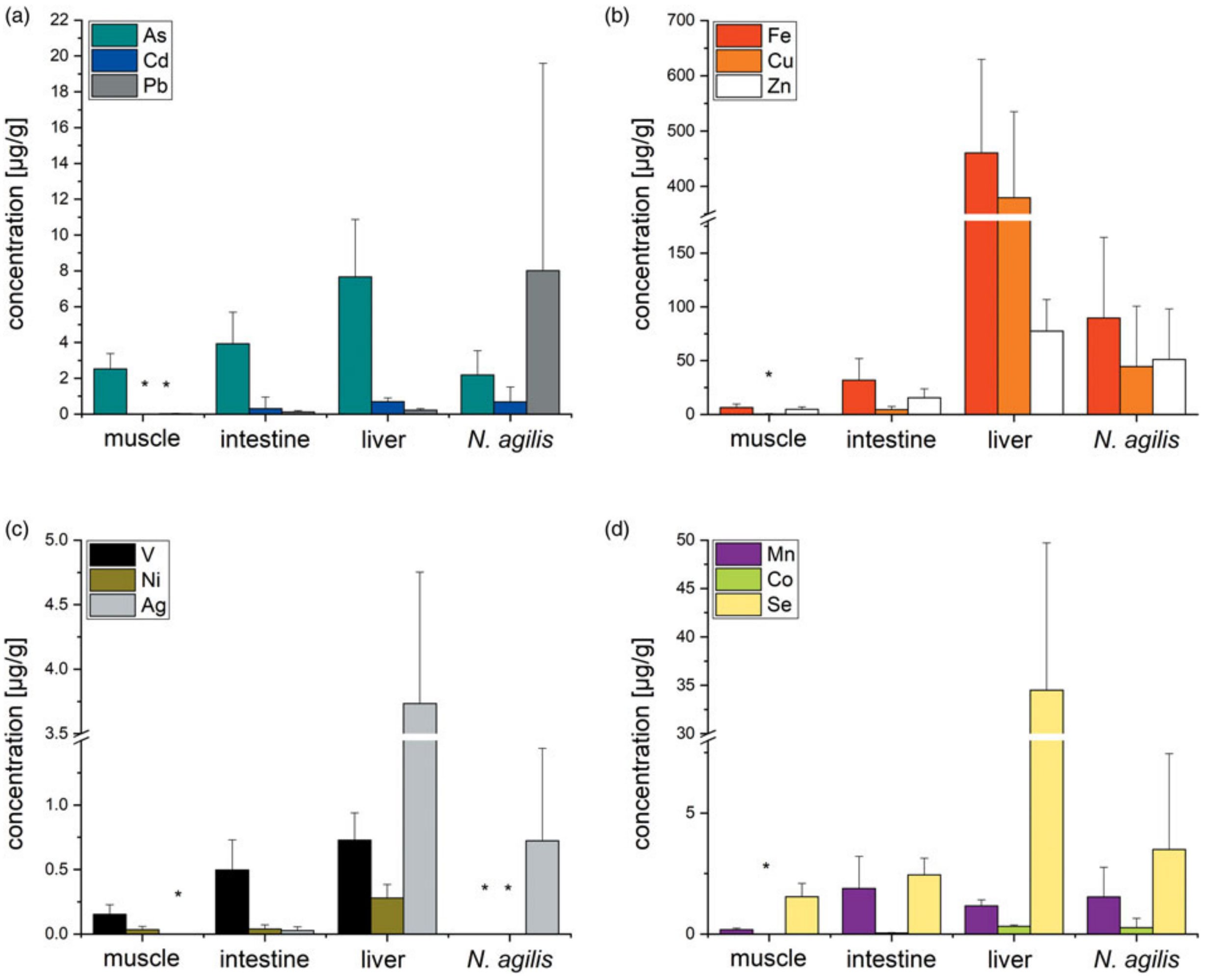

Fig. 1. Mean (± standard deviation) element concentrations (A–D) in tissues of the grey mullet *Mugil cephalus* and its acanthocephalan *Neoechinorhynchus agilis*. *Concentrations of Cu, Cd, Pb and Ag in muscle samples and concentrations of Ni and V in *N. agilis* are not displayed as they were below the detection limit.

to low. This distribution of concentration patterns in fish tissues was very similar to that found in *M. cephalus*, collected in the Ghar el Melah Lagoon in Tunisia (Chouba *et al.*, 2007). According to this study, the liver accumulated the greatest amount of Cd, mercury and Pb compared to the rest of the organs and tissues of the fish analysed. This finding does not seem surprising since many previous studies have shown the great importance of the liver in detoxifying the organisms of different pollutants (Sures & Taraschewski, 1995; Storelli *et al.*, 2006; Yilmaz, 2009; Al-Hasawi, 2019). The liver is a metabolically active organ that stores and removes enormous amounts of metals, in contrast to the non-active tissue of the muscle that has a low metal-carrying capacity (Squadrone *et al.*, 2013). During our work, the concentrations of most elements in the muscle were lower compared to those in the intestine and the liver, and according to FAO (1983) can be considered as appropriate for human consumption.

During our survey, we were interested in studying the accumulating capacity of different elements not only in the muscle, liver and intestine of the two mullet species *M. cephalus* and *C. ramada*, but also in their associated acanthocephalan parasites. The parasite *N. agilis* was found to be able to accumulate significantly higher amounts of toxic elements (such as V, Pb and Ni) compared to different host tissues.

With respect to the BCFs, in both host–parasite systems examined, the acanthocephalan demonstrated significant accumulation capacity for Ag (up to 2714 times more than muscle), and for Pb (up to 32 times more than the liver). This great ability of acanthocephalans to accumulate non-essential elements has been demonstrated in previous studies conducted on the acanthocephalan *Pomphorhynchus laevis* found in its host *Squalius cephalus* collected from the Ruhr River (Sures *et al.*, 1994a) as well as in other studies on the same parasite in the barbel fish *Barbus barbus* from the Danube River in Bulgaria (Nachev *et al.*, 2010). The results found during our investigations are in agreement with the aforementioned studies, with the exception that the accumulating capacity of Pb in the acanthocephalans of the chub and the barbel was greater than that found in *N. agilis* of the two examined mullets. This may be related to differences in metal concentrations in the different aquatic environments surveyed as well as differences in their salinity. In fact, lagoon environments have a greater salinity than freshwater environments, and this can have an effect on metal bioaccumulation in aquatic organisms. Water salinity has been shown to affect the biological availability of trace metal elements in sediments as well as in aquatic organisms (Du Laing *et al.*, 2008). For example, a study on the flounder *Platichthys flessus* from marine habitats (acclimated to sea water) and from fresh water demonstrated that salinity

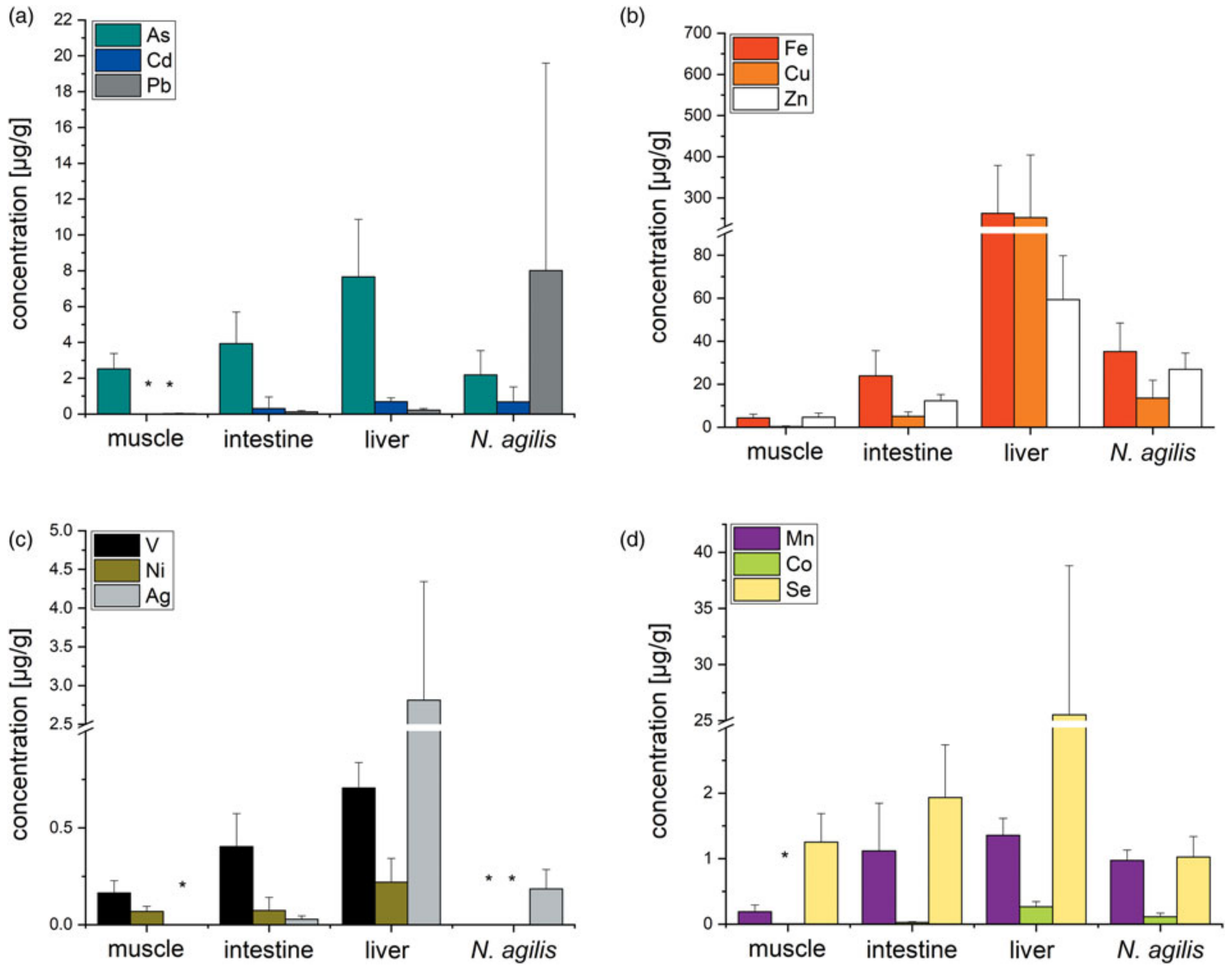

Fig. 2. Mean (± standard deviation) element concentrations (A–D) in organs of *Chelon ramada* and its intestinal parasite *Neoechinorhynchus agilis*. *Concentrations are not displayed as they were below the detection limit.

Table 4. Bioconcentration factors $C_{[N.\ agilis]}$ / $C_{[M.\ cephalus\ \mathrm{tissue}]}$ for *Neoechinorhynchus agilis* calculated with respect to different host (*Mugil cephalus*) tissues.

	Muscle	Intestine	Liver
Element	$C_{[N.\ agilis]}$ / $C_{[\mathrm{muscle}]}$	$C_{[N.\ agilis]}$ / $C_{[\mathrm{Intestine}]}$	$C_{[N.\ agilis]}$ / $C_{[\mathrm{liver}]}$
Ag	413	27	1.0
As	0.01	0.2	0.03
Cd	495	2.4	0.01
Co	50	6.1	0.08
Cu	104	10	0.02
Fe	14	2.6	0.2
Mn	8.0	1.0	1.3
Ni	25	22	2.0
Pb	390	69	35
Se	2.1	1.6	0.02
V	14	4.3	3.5
Zn	10	3.2	0.4

Table 5. Bioconcentration factors $C_{[N.\ agilis]}$ / $C_{[C.\ ramada\ \mathrm{tissue}]}$ for *Neoechinorhynchus agilis* calculated with respect to different host (*Chelon ramada*) tissues.

	Muscle	Intestine	Liver
Element	$C_{[N.\ agilis]}$ / $C_{[\mathrm{muscle}]}$	$C_{[N.\ \mathrm{agilis}]}$ / $C_{[\mathrm{intestine}]}$	$C_{[N.\ agilis]}$ / $C_{[\mathrm{liver}]}$
Ag	823	51	1.1
As	0.7	0.3	0.1
Cd	662	8.7	2. 5
Co	48	8.8	0.9
Cu	279	17	0.6
Fe	9.5	2.7	0.2
Mn	13	2.3	1.7
Ni	8.4	7.9	3.4
Pb	198	63	40
Se	3.4	2.9	0.4
V	5.5	2.0	1.3
Zn	9.2	3.8	1.1

lowered the accumulation of metals in fish (Stagg & Shuttleworth, 1982). Fish in sea water had lower Cu concentrations than those in fresh water (Stagg & Shuttleworth, 1982). Another study by Reynolds *et al.* (2018) revealed similar results, indicating that following an increase in salinity in the fish habitat, a decrease in the rate of accumulation of metals can be observed. These authors have empirically proven that the BCFs in fish tissue are much higher in the low-salinity environment. This may explain the differences in levels of accumulation of metals between lagoon-dwelling acanthocephalans and other freshwater conspecifics. Additionally, the taxonomic position of *Neoechinorhynchus rutilii* might affect its metal accumulation capacity. Until now, most of the published data on acanthocephalans considered members of the Palaeacanthocephala, whereas studies on eoacanthocephalans are scarce. There are only a few studies that have addressed metal accumulation in eoacanthocephalan species (e.g. *Paratenuisentis ambiguus* – Sures *et al.*, 1994b, 2003; Zimmermann *et al.*, 1999, 2005). Although these studies also showed higher metal concentrations in *P. ambiguus* compared to its eel host, BCFs were much lower as compared to palaeacanthocephalans. It can be hypothesized that the differences in tegument organization of eoacanthocephalans and palaeacanthocephalans might be responsible for differences in metal accumulation. However, the comparison of metal accumulation in eoacanthocephalans and palaeacanthocephalans is still based on an insufficient number of studies in order to draw any conclusion.

With regard to the acanthocephalan *N. agilis*, compared to its hosts *M. cephalus* and *C. ramada*, a considerable uptake of Ag by the intestinal parasite was observed. This resembles the result described previously in the study of metal accumulation in the marine acanthocephalan *Aspersentis megarhynchus* from *Notothenia coriiceps* collected from presumably low-polluted areas in the Antarctic (Sures & Reimann, 2003). According to this study, Ag levels in the acanthocephalan examined were approximately 20 times higher than in the intestine of the fish and 36 times higher than in the liver. The presence of high levels of this non-essential element in the present study indicates an anthropogenic pollution threat of the brackish environment surveyed. Additionally, significant differences in the accumulation rate were observed between the liver and the acanthocephalan in both host species for V, Ni and especially Cd and Pb. For these last two elements, considerable quantities have been recorded in *N. agilis*. This result was in accordance with other studies conducted on the two acanthocephalans *Acanthocephalus lucii* and *P. laevis* in their hosts (perch and chub), indicating the high capacity of these parasites to accumulate Cd and Pb compared to their hosts (up to 2700 times more than muscle) (Sures & Taraschewski, 1995; Sures & Siddall, 1999; Sures & Siddall, 2001). In our case, the significant Pb contamination in the Ichkeul Lagoon, in the tissue of the mullets and their acanthocephalan, was expected due to the presence of an old lead mine and a metallurgical company 'Elfouledh' near the lagoon. The latter is responsible for the release of large quantities of Pb and Cd, which considerably increased the pollution levels in the lagoon (water column, sediments) (Ouchir *et al.*, 2016). Regarding the rest of the metals, although their source remains unclear, their presence can be attributed mainly to urban inputs, the influx of agricultural products and the discharging of wastewater through wadis that flow into the lagoon (Ouchir *et al.*, 2016; Ben Salem *et al.*, 2017).

In conclusion, the results found during the present study confirm the possibility of the use of parasites, mainly acanthocephalans, in the bio-monitoring of aquatic ecosystems. Host–parasite systems can be considered a promising tool for the assessment of metal pollution in brackish or marine environments, where the bioaccumulation potential of parasites remains less investigated in comparison with freshwater ecosystems (summarized by Sures *et al.*, 2017). Therefore, it is highly recommended that future studies consider the parasite load of aquatic organisms in order to increase the effectiveness of environmental monitoring programs.

The present study addresses for the first time the capacity of the absorption and accumulation of elements in the tissues of *M. cephalus* and *C. ramada* mullets and their acanthocephalan *N. agilis*. The results are preliminary and support future research to elucidate the most effective bio-indicative properties in brackish environments that are poorly studied.

Acknowledgements. The authors would like to thank Mr Nejib Chaabouni and all of the other fishermen and management staff from 'Société Tunisie Lagunes', Ichkeul Lagoon, Tinja, for providing live fish.

Financial support. None.

Conflicts of interest. None.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals.

References

Al-Hasawi ZM (2019) Environmental parasitology: intestinal helminth parasites of the siganid fish *Siganus rivulatus* as bioindicators for trace metal pollution in the Red Sea. *Parasite* **26**, 12.

Annabi A, El Mouadeb R and Herrel A (2017) Distinctive accumulation patterns of heavy metals in *Sardinella aurita* (Clupeidae) and *Mugil cephalus* (Mugilidae) tissues. *Environmental Science and Pollution Research* **25**, 2623–2629.

Ben M'barek N (2001) *Etude de l'écosystème du lac Ichkeul et de son bassin versant. 19 Caractéristiques physiques et géochimiques des eaux et des sédiments.* Doctoral thesis, Université de Tunis II, Tunisia, 235 pp.

Ben Salem F, Ben Said O, Mahmoudi E, Duran R and Monperrus M (2017) Distribution of organic contamination of sediments from Ichkeul Lake and Bizerte Lagoon, Tunisia. *Marine Pollution Bulletin* **123**, 329–338.

Birk S, Chapman D, Carvalho L, *et al.* (2020) Impacts of multiple stressors on freshwater biota across spatial scales and ecosystems. *Nature Ecology & Evolution* **4**, 1060–1068.

Bone Q and Moore R (2008) *Biology of fishes*. 3rd edn. Abingdon, Oxon, Taylor & Francis.

Chouba L, Kraiem M, Njimi W, Tissaoui CH, Thompson JR and Flower RJ (2007) Seasonal variation of heavy metals (Cd, Pb and Hg) in sediments and in mullet, *Mugil cephalus* (Mugilidae), from the Ghar el Melh Lagoon (Tunisia). *Transitional Waters Bulletin* **4**, 45–52.

Du Laing G, De Vos R, Vandecasteele B, Lesage E, Tack FMG and Verloo MG (2008) Effect of salinity on heavy metal mobility and availability in intertidal sediments of the Scheldt estuary. *Estuarine, Coastal and Shelf Science* **77**, 589–602.

FAO (1983) Compilation of legal limits for hazardous substances in fish and fishery products. *FAO Fishery Circular, Food and Agriculture Organization* **464**, 5–100.

Fernandes C, Fontainhas-Fernandes A, Peixoto F and Salgado MA (2007) Bioaccumulation of heavy metals in *Liza saliens* from the Esomriz Paramos Coastal Lagoon, Portugal. *Ecotoxicology and Environmental Safety* **66**, 426–431.

Genç TO and Yilmaz F (2017) Metal accumulations in water, sediment, crab (*Callinectes sapidus*) and two fish species (*Mugil cephalus* and *Anguilla Anguilla*) from the Köyceğiz lagoon system-Turkey: an index analysis approach. *Bulletin of Environmental Contamination and Toxicology* **99**(1), 173–181.

Hamza-Chaffai A (2014) Usefulness of bioindicators and biomarkers in pollution biomonitoring. *International Journal of Biotechnology for Wellness Industries* **3**, 19–26.

Jithendran KP and Kannappan S (2010) A short note on heavy infection of acanthocephalan worm (Neoechinorhynchusagilis) in grey mullet, *Mugil cephalus*. *Journal of Parasitological Diseases* **34**, 99–101.

Kefi F, Mleiki A, Maâtoug Béjaoui J and Trigui El Menif N (2016) Seasonal variations of trace metal concentrations in the soft tissue of *Lithophaga Lithophaga* collected from the Bizerte Bay (Northern Tunisia, Mediterranean Sea). *Journal of Aquaculture Research & Development* **7**, 6.

Merian E, Anke M, Ihnat M and Stoeppler M (2004) *Elements and their compounds in the environment*. 2nd edn. Weinheim, Wiley-VCH.

Nachev M and Sures B (2015) Environmental parasitology: parasites as accumulation bioindicators in the marine environment. *Journal of Sea Research* **113**, 45–50.

Nachev M, Zimmermann S, Rigaud T and Sures B (2010) Is metal accumulation in *Pomphorhynchus laevis* dependent on parasite sex or infrapopulation size? *Parasitology* **137**(12), 39–48.

Olsson PE, Kling P and Hogstrand C (1998) Mechanisms of heavy metal accumulation and toxicity in fish. pp. 321–350 *in* WJ Langston and M Bebianno (*Eds*) *Metal metabolism in aquatic environments*. London, Chapman and Hall.

Ouchir N, Ben Aissa L, Boughdiri M and Aydi A (2016) Assessment of heavy metal contamination status in sediments and identification of pollution source in Ichkeul Lake and rivers ecosystem, Northern Tunisia. *Arab Journal of Geoscience* **9**, 539.

Reynolds EJ, Smith DS, Chowdhury MJ and Hoanga TC (2018) Chronic effects of lead exposure on topsmelt fish (*Atherinops affinis*): influence of salinity, organism age, and relative sensitivity to other marine species. *Environmental Toxicological Chemistry* **37**, 2705–2713.

Rosenberg DM and Resh VH (1993) Introduction to freshwater biomonitoring and benthic macroinvertebrates. pp. 1–9 *in* Rosenberg DM and Resh VH (*Eds*) *Freshwater biomonitoring and benthic macroinvertebrates*. New York, Chapman/Hall.

Squadrone S, Prearo M, Brizio P, Gavinelli S, Pellegrino M, Scanzio T, Guarise S, Benedetto A and Abete MC (2013) Heavy metals distribution in muscle, liver, kidney and gill of European catfish (*Silurus glanis*) from Italian rivers. *Chemosphere* **90**, 358.

Stagg RM and Shuttleworth TJ (1982) The accumulation of copper in *Platichthys flesus* L. and its effects on plasma electrolyte concentrations. *Journal of Fish Biology* **20**, 491–500.

Storelli MM, Barone G, Storelli A and Marcotrigiano GO (2006) Trace metals in tissues of mugilids (*Mugil auratus*, *Mugil capito*, and *Mugil labrosus*) from the Mediterranean Sea. *Bulletin of Environmental Contamination and Toxicology* **77**, 43–50.

Sures B (2003) Accumulation of heavy metals by intestinal helminths in fish: an overview and perspective. *Parasitology* **126**, 53–60.

Sures B (2004) Environmental parasitology: relevancy of parasites in monitoring environmental pollution. *Parasitology* **20**, 170–177.

Sures B (2005) Effects of pollution on parasites, and use of parasites in pollution monitoring. pp. 421–425 *in* Rohde K (*Ed.*) *Marine parasitology*. Collingwood, CSIRO Publishing.

Sures B and Reimann N (2003) Analysis of trace metals in the Antarctic host parasite system *Motothenia coriiceps* and *Aspersentis megarhynchus* (Acanthocephala) caught at King George Island, South Shetland Islands. *Polar Biology* **26**, 680–686.

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

Sures B and Siddall R (2001) Comparison between lead accumulation of *Pomphorhynchus laevis* (Palaeacanthocephala) in the intestine of chub (*Leuciscus cephalus*) and in the body cavity of goldfish (*Carassius auratus auratus*). *International Journal for Parasitology* **31**, 669–673.

Sures B and Taraschewski H (1995) Cadmium concentrations of two adult acanthocephalans (*Pomphorhinchus leavis*, *Acanthocephalus lucii*) compared to their fish host and cadmium and lead levels in the larvae of *A. Lucii* compared to their crustacean host. *Parasitology Research* **81**, 494–497.

Sures B, Taraschewski H and Jackwerth E (1994a) Lead accumulation in Pomphorhynchus laevis and its host. *Journal of Parasitology* **80**, 355–357.

Sures B, Taraschewski H and Jackwerth E (1994b) Lead content of Paratenuisentis ambiguus (Acanthocephala), Anguillicola crassus (Nematodes) and their host Anguilla anguilla. *Diseases of Aquatic Organisms* **19**, 105–107.

Sures B, Taraschewski H and Rydlo M (1997) Intestinal fish parasites as heavy metal bioindicators: a comparison between *Acanthocephalus lucii* (Palaeacanthocephala) and the zebra mussel, *Dreissena polymorpha*. *Bulletin of Environmental Contamination and Toxicology* **59**, 14–21.

Sures B, Siddall R and Taraschewski H (1999) Parasites as accumulation indicators of heavy metal pollution. *Parasitology Today* **15**, 16–21.

Sures B, Zimmermann S, Sonntag C, Stüben D and Taraschewski H (2003) The acanthocephalan Paratenuisentis ambiguus as a sensitive indicator of the precious metals Pt and Rh emitted from automobile catalytic converters. *Environmental Pollution* **122**, 401–405.

Sures B, Nachev M, Pahl M, Grabner D and Selbach C (2017) Parasites as drivers of key processes in aquatic ecosystems: facts and future directions. *Experimental Parasitology* **180**, 141–147.

Tkach IV, Sarabeev VL and Shvetsova LS (2014) Taxonomic status of *Neoechinorhynchus agilis* (Acanthocephala, Neoechinorhynchidae), with a description of two new species of the genus from the Atlantic and Pacific mullets (Teleostei, Mugilidae). *Vestnik Zoologii* **48**, 291–306.

Waltham NJ, Teasdale PR and Connolly RM (2013) Use of flathead mullet (*Mugil cephalus*) in coastal biomonitor studies: review and recommendations for future studies. *Marine Pollution Bulletin* **69**, 195–205.

Yazidi A, Saidi S, Ben Mbarek N and Darragi F (2017) Contribution of GIS to evaluate surface water pollution by heavy metals case of Ichkeul lake (Northern Tunisia). *Journal of African Earth Sciences* **134**, 166–173.

Yilmaz AB (2009) The comparison of heavy metal concentrations (Cd, Cu, Mn, Pb and Zn) in tissues of three economically important fish (*Anguilla Anguilla*, *Mugil cephalus* and *Oreochromis niloticus*) inhabiting Köycegiz Lake-Mugla (Turkey). *Turkish Journal of Science & Technology* **4**(1), 7–15.

Zimmermann S, Sures B and Taraschewski H (1999) Experimental studies on lead accumulation in the eel specific endoparasites *Anguillicola crassus* (Nematoda) and *Paratenuisentis ambiguus* (Acanthocephala) as compared with their host, *Anguilla Anguilla*. *Archives of Environmental Contamination and Toxicology* **37**, 190–195.

Zimmermann S, Messerschmidt J, Von Bohlen A and Sures B (2005) Uptake and bioaccumulation of platinum group metals (Pd, Pt, Rh) from automobile catalytic converter materials by the zebra mussel (*Dreissena polymorpha*). *Environmental Research* **98**, 203–209.