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VERSITA

The system *Tetrabothrius bassani* (Tetrabothriidae)/ *Morus bassanus* (Sulidae) as a bioindicator of marine heavy metal pollution

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Abstract

Helminths are known to accumulate higher amounts of certain elements than their hosts. The present study assesses the accumulation of heavy metals in *Tetrabothrius bassani* and in its host, the Atlantic gannet (*Morus bassanus*) found dead due to by-catch along the seashore in the centre of Portugal. Samples of kidney, liver and pectoral muscle of 23 infected gannets, as well as specimens of *T. bassani* were analysed for As, Cd, Cr, Cu, Hg, Mn, Pb, Se and Zn by ICP-MS. The evidenced lower concentrations of Cr and Pb in tissues of gannets in comparison to an earlier study performed in the same area may reveal a change in the diet of *M. bassanus* between both study periods. The highest bioaccumulation factor was obtained for Cd with a 12.7-times higher concentration in the cestode than in gannet muscle. Lead concentration in *T. bassani* was 6.9-times higher than in kidney tissue, 8.5-times higher than in muscle and 9.5-times higher than in liver of *M. bassanus*. The cestode/seabird system *T. bassani*/*M. bassanus* can be considered a promising bioindicator system to monitor environmental Cd and Pb pollution in marine ecosystems.

Keywords

Tetrabothrius bassani, *Morus bassanus*, heavy metals, helminth/seabird system

Introduction

Studies on the interactions between pollution and parasites have been increasing over the last years and it is known that some helminths are able to accumulate heavy metals in concentrations higher than their hosts (Sures 2003, 2004). High bioaccumulation factors have been reported in parasite/host systems involving acanthocephalans of fish (Sures and Taraschewski 1995, Sures and Siddall 1999, Sures *et al.* 2005, Brázová *et al.* 2012, Jankovska *et al.* 2012). Cestodes have been also found to accumulate relatively high amounts of heavy metals and therefore several cestode/fish systems were also proposed as useful indicators of heavy metal pollution in the aquatic environment (Jirsa *et al.* 2008, Eira *et al.* 2009, Brázová *et al.* 2012).

However, data on parasite-host systems as bioindicators of heavy metal pollution in the marine environment are still scarce considering that the available information mainly concerns freshwater or estuarine ecosystems. In fact, the available data on parasites of marine hosts refer to only a few cestode and nematode species. The studied cestodes are *Bothriocephalus scorpii* infecting turbots (*Scophthalmus maximus*) from the coast of Poland (Sures *et al*. 1997) and *Anthobothrium* sp. and *Paraorigmatobothrium* sp. parasitizing the shark (*Carcharhinus dussumieri*) from the Persian Gulf (Malek *et al*. 2007). The studied nematodes include *Anisakis simplex s.l.* infecting cetaceans (Pascual and Abollo 2003), *Hysterothylacium aduncum* parasitizing *Sparus aurata* in the Iskenderun Bay (Dural *et al.* 2011) and *Hysterothylacium* sp. type MB larvae infecting *Trichiurus lepturus* in the Gulf of Oman (Khaleghzadeh-Ahangar *et al.* 2011).

Heavy metals in marine environments emerge through different pathways (waste products, industrial activities and natural processes). Their biomagnification along the food chain

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is a major concern in marine environments, increasing the potential for human exposure (Mulvad *et al.* 1996, Ross 2000, Li *et al.* 2010). In this context, other than fish and cetaceans, seabirds have been often used as bioindicators of heavy metals in the marine environment because they are long-lived and feed at different distances from land, and because of their high trophic position, exhibiting different trophic levels (Hahn *et al.* 1993, Burger and Gochfeld 2000, Savinov *et al.* 2003, Mallory *et al.* 2010). Contrarily, the evaluation of parasite-seabird host systems in heavy metal pollution biomonitoring is still scarce. Systems involving birds living in freshwater ecosystems have already been proposed (Barus *et al.* 2000, Tenora *et al.* 2002), but there are no data concerning marine birds except for two cormorant species (*Phalacrocorax carbo* and *P. auritus*) which are more coastal rather than truly oceanic birds being used to colonise inland waters (Barus *et al.* 2001, Robinson *et al.* 2010).

The purpose of this study was to assess the accumulation of trace elements in *Tetrabothrius bassani* Burt, 1978 (Cestoda: Tetrabothriidae) in relation to its host *Morus bassanus* Linnaeus, 1758 (Pelecaniformes: Sulidae), and evaluate this system as the first cestode/seabird bioindicator for heavy metals pollution in the marine environment.

Materials and Methods

Twenty-three Atlantic gannets (*M. bassanus*) parasitized by *T. bassani* were used to perform the present study. All individuals were found dead due to by-catch (accidental capture by fishing gear) in 2007 and 2008 in the central coast of Portugal (40º13´14″ N and 8º53´31″ W). Cause of death was confirmed during necropsies. Apart entangled nets, fractures, abrasions and cuts in wings and legs, all gannets presented good body condition, undigested fish in proventriculi and haemorrhagic lungs. The digestive tracts of gannets were scanned for helminths using a stereomicroscope with the help of stainless steel instruments and Milli-Q water. At least one individual of *T. bassani* from each parasitized *M. bassanus*, as well as respective samples of kidney, liver and pectoral muscle tissues were collected and stored in glass vials and deep-frozen until posterior analysis.

Kidney, liver and pectoral muscle samples of the 23 gannets studied (± 150 mg wet weight) as well as the respective *T. bassani* specimens were individually digested in Teflon vessels with HNO_3 (2 ml) and H_2O_2 (1 ml) (Merck, Suprapure) at 90ºC in an oven and left overnight. All material used in the digestion process was thoroughly acid-rinsed. After digestion, samples were diluted with Milli-Q water and then analysed for As, Cd, Cr, Cu, Hg, Mn, Pb, Se and Zn in an inductively coupled plasma mass spectrometer (Perkin Elmer Elan 6000). The analytical procedure was checked using standard reference material: *Squalus acanthias* liver (DOLT-3) and *Squalus acanthias* muscle (DORM-2) both provided by the National Research Council of Canada. Analytical blanks were also used to determine the detection limits.

Concentrations of most of the evaluated elements were not normally distributed, thus Kruskal-Wallis and post-hoc Dunn's tests were used. All analyses were performed in Prism 5.0 (Graph Pad Software Inc). A significance level of $P < 0.05$ was applied for all tests. The bioaccumulation factors were determined as the ratio of the element concentration in the parasites to that in different host tissues ($BF = C_{[parasite]}/C_{[host\ tissue]}$) according to Sures *et al.* (1999).

Results

Tetrabothrius bassani was the only intestinal helminth present in the analysed gannets. The detection limits for the evaluated elements were inferior to 1 ng ml^{-1} except for Zn (5.8 ng ml^{-1}). With respect to the analytical procedure, considering the elements present in the respective reference material, ICP-MS analysis revealed accuracy rates ranging between 85.1% for Cu and 105.3% for Se. Mean values (± standard errors) and ranges of all element concentrations found in kidney, liver and muscle of *M. bassanus* and those found in *T. bassani* are summarized in Table I.

Table I. Mean element concentration (± standard error, SE) and range values in soft tissues of *Morus bassanus* and in *Tetrabothrius bassani* (µg g^{-1} wet weight) from Portugal

	Kidney		Liver		Muscle		Cestode	
	Mean (SE)	Range	Mean (SE)	Range	Mean (SE)	Range	Mean (SE)	Range
As	0.528 (0.046)	0.258–1.215	0.465 (0.052)	0.145–1.188	0.707 (0.058)	0.314–1.432	0.811 (0.077)	0.096–1.789
Cd	1.375 (0.352)	0.004–5.882	0.268 (0.077)	0.013–1.186	0.009 (0.002)	0.001–0.025	0.114 (0.024)	0.025–0.537
Cr	0.378 (0.029)	0.110–0.635	0.406 (0.061)	0.082–1.576	0.621 (0.063)	0.215–1.682	0.759 (0.128)	0.160–3.157
Cu	4.545 (0.472)	2.425–12.685	7.377 (0.625)	2.797–15.231	4.290 (0.345)	2.045–9.077	2.065 (0.213)	0.017–4.503
Hg	0.532 (0.067)	0.138–1.608	0.851 (0.104)	0.242–2.108	0.242 (0.022)	0.053–0.527	0.315 (0.027)	0.022–0.600
Mn	1.694 (0.151)	0.375–3.175	4.569 (0.461)	1.582–10.787	0.976 (0.528)	0.116–12.487	3.294 (0.984)	0.200–22.740
Pb	0.036 (0.006)	0.015–0.110	0.026 (0.005)	0.015–0.130	0.029 (0.063)	0.014–0.082	0.247 (0.165)	0.009–3.904
Se	5.185 (0.787)	1.540–14.667	4.531 (0.739)	1.028–13.334	1.364 (0.250)	0.295–5.345	1.048 (0.121)	0.168–2.753
Zn	36.489 (2.901)	24.241–78.298	59.631 (8.265)	22.026–152.772	30.781 (2.610)	15.127–63.698	70.931 (7.809)	6.194–188.797

Table II. Bioaccumulation factors (range), $[C]_{parasite}$ / $[C]_{host\ tissue}$, evidenced in *Tetrabothrius bassani* in relation to *Morus bassanus* tissues

	BF_{Kidney}	BF_{Liver}	BF_{Muscle}
As	1.5 (0.2–3.9)	1.7 (0.4–4.6)	< 1
Cd	< 1	< 1	12.7 (1.3–106.3)
Cr	2.0 (0.4–9.0)	1.9 (0.3–6.8)	< 1
Mn	< 1	< 1	3.4 (0.2–69.3)
Pb	6.9 (0.1–60.7)	9.5 (0.4–237.1)	8.5 (0.2–57.1)
Zn	1.9 (0.2–7.7)	< 1	2.3 (0.3–5.6)

Some trace elements presented significantly higher average concentrations in seabird tissues than in the cestode such as Cu in liver, kidney and muscle tissue, Se in liver and kidney, and Mn in liver (Kruskal-Wallis, $49.4 < H < 52.06$, $P < 0.0001$; Dunn's test, $P < 0.001$). There were also significantly higher average concentrations of Cd in gannet's kidney and Hg in liver (Kruskal-Wallis, $H = 45.61$ and $H = 43.29$, $P < 0.0001$, respectively) in comparison to those in cestodes (Dunn's test, $P < 0.001$). However, average concentrations of As, Cr, Mn and Zn in *T. bassani* were significantly higher than those found in some *M. bassanus* soft tissues (Kruskal-Wallis, $19.5 < H < 49.4$, $P < 0.0002$). This is the case of As and Cr concentrations in the cestode when compared to values in kidney and liver tissues (Dunn's test, $P < 0.05$). Also the concentrations of Mn in seabird muscle tissue and the concentrations of Zn in kidney and muscle tissue were significantly lower than those in the cestode ($P < 0.05$).

The highest significant bioaccumulation factor (BF) was found for Cd, with a 12.7-times higher average concentration in the cestode in comparison to gannet muscle (Kruskal-Wallis, $H = 45.61$, $P < 0.05$) with a maximum value of 106.3 (Table II). High average BFs were obtained for Pb with respect to kidney (BF = 6.9), liver (BF = 9.5) and muscle (BF = 8.5) (Table II). However, the Pb concentrations in the cestode were not considered significantly higher than those in gannet's tissues (Kruskal-Wallis, $H = 5.32$, $P = 0.15$).

Discussion

In a previous study focused on non-parasitized *M. bassanus* collected during the period 2003–2006 in the same area of Portugal, among other toxic elements, a high concentration of mercury was reported, which could represent an early warning indication of pollution (Mendes *et al.* 2008) since the concentrations of mercury in gannets were roughly above the minimum concentration for adverse effects in birds (Eisler 1987).

Mendes *et al.* (2008) report dry weight-based trace element concentrations and humidity percentage values in kidney, liver and muscle samples of *M. bassanus*, thus allowing us to transform their data into wet weight values. We were then able to verify that most element concentrations in gannets presented in our study are within the range of the values reported by Mendes *et al.* (2008). However, our results indicate relatively lower concentrations of Cr and Pb in kidney, liver and muscle samples and also higher concentrations of Hg in liver in comparison to liver tissue in any of the gannets' age classes reported in Mendes *et al.* (2008). Even though a high concentration of Hg is expected in such predatory seabirds, our results seem to corroborate the earlier study on gannets with respect to the above-mentioned potential Hg pollution. In general the relative differences between the values presently reported and those from birds evaluated by Mendes *et al.* (2008) may result from a different use of food resources, considering diet's confirmed role as a major contamination pathway in marine vertebrates (Kim *et al.* 1998).

An earlier assessment of the cestode/fish system *Proteocephalus macrocephalus*/*Anguilla anguilla* from an estuarine environment in Portugal (Ria de Aveiro) suggested that the cestode accumulated Cr and Ni while the concentrations of Cr in eel livers and Ni in eel kidneys were comparatively lower (Eira *et al*. 2009). Lower metal contents in fish hosting helminth parasites in comparison to fish without parasites have been described in other studies (Malek *et al*. 2007, Brázová *et al*. 2012). Even though the toxic element concentrations reported in the present set of gannets were not compared to those from another set of not infected specimens, the present data concur with the possible antagonistic effect of simultaneously occurring parasites and pollutants, since parasites seem to act as heavy metal filters or sinks removing them from host tissues (Sures 2008). In fact, considering the obtained bioaccumulation factors between *T. bassani* and *M. bassanus*, relevant values were obtained for Cd and Pb. With respect to Cd a very high average BF was registered in relation to seabird muscle ($BF_{Cd} = 12.7$) with a maximum BF_{Cd} of 106.3. This value is much higher than that found for another helminth/bird system in a previous study on *Raillietina micracantha*/*Columba livia* from an urban environment (maximum $BF_{Cd,muscle} = 57.25$) performed by Torres *et al*. (2010). Although an higher number of samples should be assessed in the future to validate the significance of the Pb bioaccumulation factors, in the present study the BFs calculated for Pb with respect to liver, kidney and muscle indicated between 7 to 10 times more Pb in *T. bassani* than in the gannet's soft tissues (Table II). Cestodes absorb nutrients across their tegument from the host intestinal lumen and therefore the higher amount of Pb in the cestode than those in kidney or liver of *M. bassanus* either indicates that seabirds may be able to excrete the circulating Pb to the intestinal lumen and/or that *T. bassani* act as sinks and therefore accumulate lead in their own tissues as occurs in acanthocephalans (Sures and Sidall 1999).

In conclusion the relative differences between the present results and those reported in Mendes *et al*. (2008), namely lower concentrations of Cr and Pb in soft tissues and higher concentrations of Hg in liver, emphasise the importance of feeding diversity on toxic element variability. Therefore, seabird dietary preferences and changes are subjects that should be considered in biomonitoring studies. The most elevated BFs were registered for Cd respect to muscle and Pb respect to liver, kidney and muscle. The present data seems to be in agreement with the possible antagonistic effects of some helminths and heavy metal pollution on host health (see Sures 2008). Although more field essays are necessary to evaluate the relationship between bioaccumulation in cestode parasites of birds and environmental trace element availability, considering the BFs obtained in the present study the system *T. bassani*/*M. bassanus* can be proposed as a promising bioindicator system to evaluate Cd and Pb in marine environments.

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