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ORIGINAL PAPER

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Analysis of trace metals in the Antarctic host-parasite system *Notothenia coriiceps* and *Aspersentis megarhynchus* (Acanthocephala) caught at King George Island, South Shetland Islands

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Abstract Concentrations of the elements Al, Ag, As, Ba, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb and Sr were analysed by high-performance quadrupole inductively coupled plasma mass spectrometry (Q-ICP-MS) in the acanthocephalan *Aspersentis megarhynchus* and in different tissues of its final host, *Notothenia coriiceps*. Infected fish were sampled at King George Island, South Shetland Islands, Antarctica. Most of the elements were found at significantly higher concentrations in the acanthocephalan than in muscle, liver and intestine of its host. Only Fe was concentrated in fish liver to a significantly higher level than in the parasite. Compared with the host tissues, the highest accumulation rates in *A. megarhynchus* were found for Pb, Cd, Ag, Ni and Cu. The acanthocephalans showed very high Ag and Pb levels, whereas the concentrations in the fish tissues were close to the detection limit. This study is the first proof that the enormous heavy-metal accumulation capacity reported for acanthocephalans from freshwater fish also occurs in acanthocephalans parasitizing marine fish. Consequently, acanthocephalans can be used to assess the occurrence and availability of even the lowest metal concentrations in all kinds of aquatic habitats, including remote areas such as the Antarctic.

Introduction

There is growing interest in assessing pollution of remote areas like the Antarctic (Kennicutt and McDonald 1996; Sanchez-Hernandez 2000). This unique marine ecosystem provides excellent opportunities to understand the effects of human perturbations on the natural environment. There is increasing concern about the wide range of anthropogenic-driven pollution, which is detected in the Antarctic ecosystem including the Antarctic fauna. Among these pollutants, organic xenobiotics, such as polychlorinated biphenyls (e.g. Focardi et al. 1995) and polyaromatic hydrocarbons (e.g. Cripps 1992; Kennicutt and McDonald 1996), but also toxic metals, are commonly detected (Viarengo et al. 1993; Zauke and Petri 1993; Focardi et al. 1995; Bargagli et al. 1996; de Moreno et al. 1997; Sanchez-Hernandez 2000; Duquesne and Riddle 2002).

When assessing the range of pollutants occurring in a given habitat, it is necessary to use the most effective bioindicators to determine those substances that are biologically available. Among these bioindicators, we include different species of crustaceans, mussels and fish (e.g. Bryan et al. 1985; Gunkel 1994; Zauke et al. 1995). In addition to their role as potential bioindicators, fish are also hosts for a wide range of parasites. Among fish parasites, acanthocephalans living in the gut are of increasing interest as potential indicators for metal pollution (reviewed in Sures 2001, 2003). In recent studies, it was demonstrated that adult acanthocephalans accumulate metals such as Pb and Cd to concentrations up to 2,700 and 400 times higher, respectively, than the levels in the muscle of their hosts (Sures 2003). Furthermore, the metal accumulation capacity of the acanthocephalan *Acanthocephalus lucii* from the intestine of perch was compared with that of the bivalve *Dreissena polymorpha* (Sures et al. 1999a), which is commonly used as a sentinel in freshwater ecosystems to asses the amount of bioavailable heavy metals and other environmental pollutants (e.g. Hendriks et al. 1998; Cope et al. 1999; Roditi et al. 2000; Zimmermann et al. 2002). The parasites were found to contain up to 167 times higher metal levels than the zebra mussel (Sures et al. 1999a). The acanthocephalans were able to simultaneously accumulate elements like Tl and Ag,

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which could not be detected in the host tissues or in the mussels. *A. lucii* was also able to accumulate Pb and Cd to a much higher degree than the isopod *Asellus aquaticus* (Sures and Taraschewski 1995), an organism that is well known for its metal accumulation capacity and resistance to toxic effects (Moldovan et al. 2001).

Although the uptake of metals has been demonstrated for acanthocephalans in limnetic habitats (Sures 2001), and elucidated carefully in laboratory studies (Sures and Siddall 1999, 2001, 2003), there were only preliminary data available on the uptake of metals by these parasites for the marine environment until now (Sures et al. 1999b). The only marine acanthocephalan that was studied in respect of its heavy metals was *Echinorhynchus gadi* from cod (*Gadus morhua*), indicating high levels of lead (Sures et al. 1999b). Thus, the aim of the present study was to asses the usefulness of acanthocephalans as heavy-metal bioindicators in the marine environment. We therefore investigated the common host-parasite system, *Notothenia coriiceps* infected with *Aspersentis megarhynchus*. By using these organisms we were able to investigate the presence and biological availability of metals around King George Island, South Shetland Islands, Antarctica.

Materials and methods

Sample collection and study area

Ten black rockcod (*N. coriiceps*, Nototheniidae; size: 35.1 ± 5.8 cm, weight: 638 ± 324 g) infected with adults of the intestinal acanthocephalan parasite *Aspersentis megarhynchus* were sampled at one sampling site (Fig. 1) at the entrance to Potter Cove, close to the Argentinean research station "Teniente Jubany" (62°14′S 58°40′W) on King George Island (South Shetland Islands). Fish were caught between January and February 1999 with fish traps at a depth of approximately 10 m. *N. coriiceps* is the dominant fish species in the area of study, both in number and biomass (Barrera-Oro and Casaux 1990). After morphometrical measurements, all specimens of *N. coriiceps* were immediately frozen at −20°C until further processing in the laboratory.

All dissection instruments were cleaned with 1% ammonium-EDTA solution and distilled water before dissecting fish. Samples of fish muscle were taken after defrosting the fish. The body cavity was opened ventrally and the entire alimentary tract was removed. The gut was prepared by sectioning along its length and removing its contents, which were carefully studied for parasites using a stereomicroscope. Acanthocephalans were removed from the contents and the intestinal wall with forceps and identified following Zdzitowiecki (1991). Specimens of *Aspersentis megarhynchus*, and samples of muscle, liver and intestinal wall were stored in plastic containers and frozen at −20°C until sample preparation.

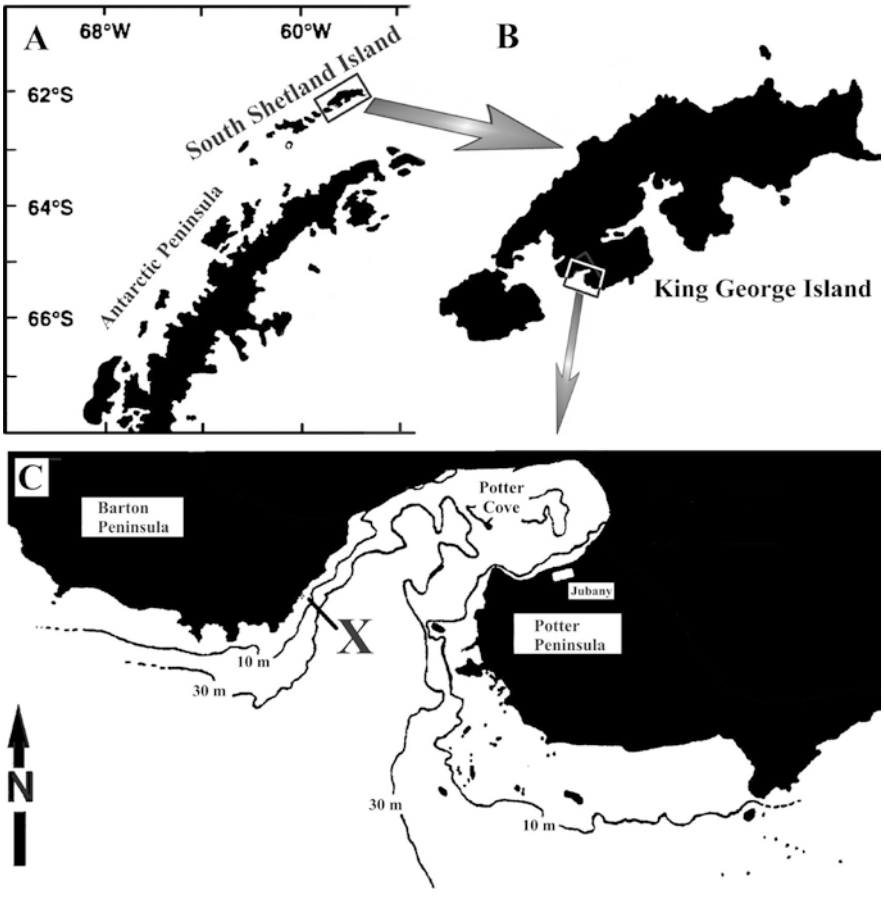

Fig. 1a–c Location of the sampling site

Sample digestion

Samples of fish muscle (without skin, scales and bones), liver and intestinal wall were homogenized with a dispersing tool (Ultra-Turrax T 25, Janke and Kunkel, Staufen, Germany). Between 100 and 200 mg of the homogenized fish tissues was digested using concentrated nitric acid (Suprapur, Merck, Darmstadt, Germany). All acanthocephalans from the gut of an individual host were pooled and treated as one sample. After each sample was weighed in a Perfluoralkoxy vessel (CEM), 1.8 ml nitric acid was added. All samples were digested using a microwave digestion procedure described earlier (Sures et al. 1995). Reference samples comprising about 100 mg (dry weight) of dogfish (*Squalus acanthias*) muscle with certified values for ten elements (DORM 2, National Research Council, Canada) were weighed and digested in the same manner as the other samples. To determine the detection limit, analytical blanks were prepared without insertion of a sample.

ICP-MS analysis

The elements Al, Ag, As, Ba, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb and Sr were analysed using a high-performance quadrupole inductively coupled plasma mass spectrometer (Q-ICP-MS, PQ ExCell, VG Elemental), operating in the normal scan modus. The instrumental operation conditions are summarized in Table 1. Before measurements were made, the microwave-digested solutions were diluted 1:5 with deionized water in order to reduce acid concentration. Element concentrations in each sample were calculated from the corresponding regression lines (correlation factor >0.99) using different dilutions of a standard solution (ICP multi-element standard solution, Merck, Germany). Detection limits were determined to be 3 times the standard deviation of the blanks measurement.

Statistical treatment

Heavy-metal concentrations in the tissues of *N. coriiceps* and its parasites were determined as $\mu g\ g^{-1}$ (wet weight) and were compared using the Friedman- and Wilcoxon-tests with significance levels of $P \leq 0.01$ for each concentration.

Results

ICP-MS analyses

The detection limits for each element, the concentrations of the metals determined from the standard reference material and the certified values for the elements of DORM 2 are summarized in Table 2. The accuracy for the elements ranged between 80 and 124%, with the highest accuracies for Co, Cr and Al. As and Ag showed the lowest accuracies, with values of 80 and 124%, respectively.

Table 1 Instrumental operating conditions for the element determination by Q-ICP-MS

Instrument:	VG Elemental PQ ExCell
Forward power:	1,350 W
Uptake rate:	1 ml/min
Scan modus:	Normal scan
Torch:	Quartz torch (Glass Expansion, Australia)
Nebuliser:	Meinhardt (Glass Expansion, Australia)
Spray chamber:	Impact bead, water cooled to 15°C
Cones:	Ni
Coolant gas:	Argon, 13.1 l/min
Auxiliary gas:	Argon, 0.75 l/min
Nebuliser gas:	Argon, 0.93 l/min
Dwell time:	100 ms

Element concentrations in *Notothenia coriiceps* and their parasites

Most of the elements investigated within this study were present at significantly higher levels ($P<0.01$) in *Aspersentis megarhynchus* than in the organs of its host (Fig. 2). Considering only the organs of *N. coriiceps*, most of the elements (Ag, B, Ba, Co, Cr, Cu, Mn, Ni, Pb and Sr) were present at the highest concentrations in the intestine, followed by the liver (Al, As, Cd, Fe and Mg), while the muscle always contained the lowest amounts of the elements. Compared with the element concentrations in the muscle of its host, *Aspersentis megarhynchus* contained significantly higher levels of nearly all the elements analysed (Fig. 2). Ag and Co were below the detection limit and thus could not be quantified in the muscle of *N. coriiceps*. The acanthocephalan also showed significantly higher levels for all elements, except for As and Fe, when compared to the liver of its host, Fe being significantly higher in fish liver than in the parasite. Although the values for B, Cd, Cu, Fe and Ni in the worm exceeded the levels in the intestine of the fish, these were the only elements not significantly concentrated in *Aspersentis megarhynchus* compared to the intestine of *N. coriiceps*.

Table 2 Element concentrations in the standard reference material DORM 2 (certified values by NRCC) and as determined by inductively coupled plasma mass spectrometry (ICP-MS); mean ± SD of eight measurements, and mean accuracies and detection limits of the ICP-MS analysis

Element	NRCC (μg/g)	ICP-MS (μg/g)	Accuracy (%)	Detection limit (ng/ml)
Ag	0.041 ± 0.013	0.051 ± 0.008	124.4	0.11
Al	10.9 ± 1.7	11.1 ± 3.0	101.7	7
As	18.0 ± 1.1	14.5 ± 1.6	80.4	0.38
B	–[a]	–	–	7.7
Ba	–	–	–	0.19
Cd	0.043 ± 0.008	0.041 ± 0.006	95.1	0.06
Co	0.182 ± 0.031	0.184 ± 0.084	100.9	0.12
Cr	34.7 ± 5.5	35.2 ± 1.9	101.5	0.56
Cu	2.34 ± 0.16	2.55 ± 0.28	109.2	3.4
Fe	142 ± 10	164 ± 12	115.6	60
Mg	–	–	–	60
Mn	3.66 ± 0.34	4.32 ± 0.24	118.0	0.13
Ni	19.4 ± 3.1	18.8 ± 2.4	96.9	1.7
Pb	0.065 ± 0.007	0.069 ± 0.027	106.5	0.25
Sr	–	–	–	2.8

[a]Element not certified in DORM 2 (dogfish muscle certified reference material for trace metals, National Research Council, Canada).

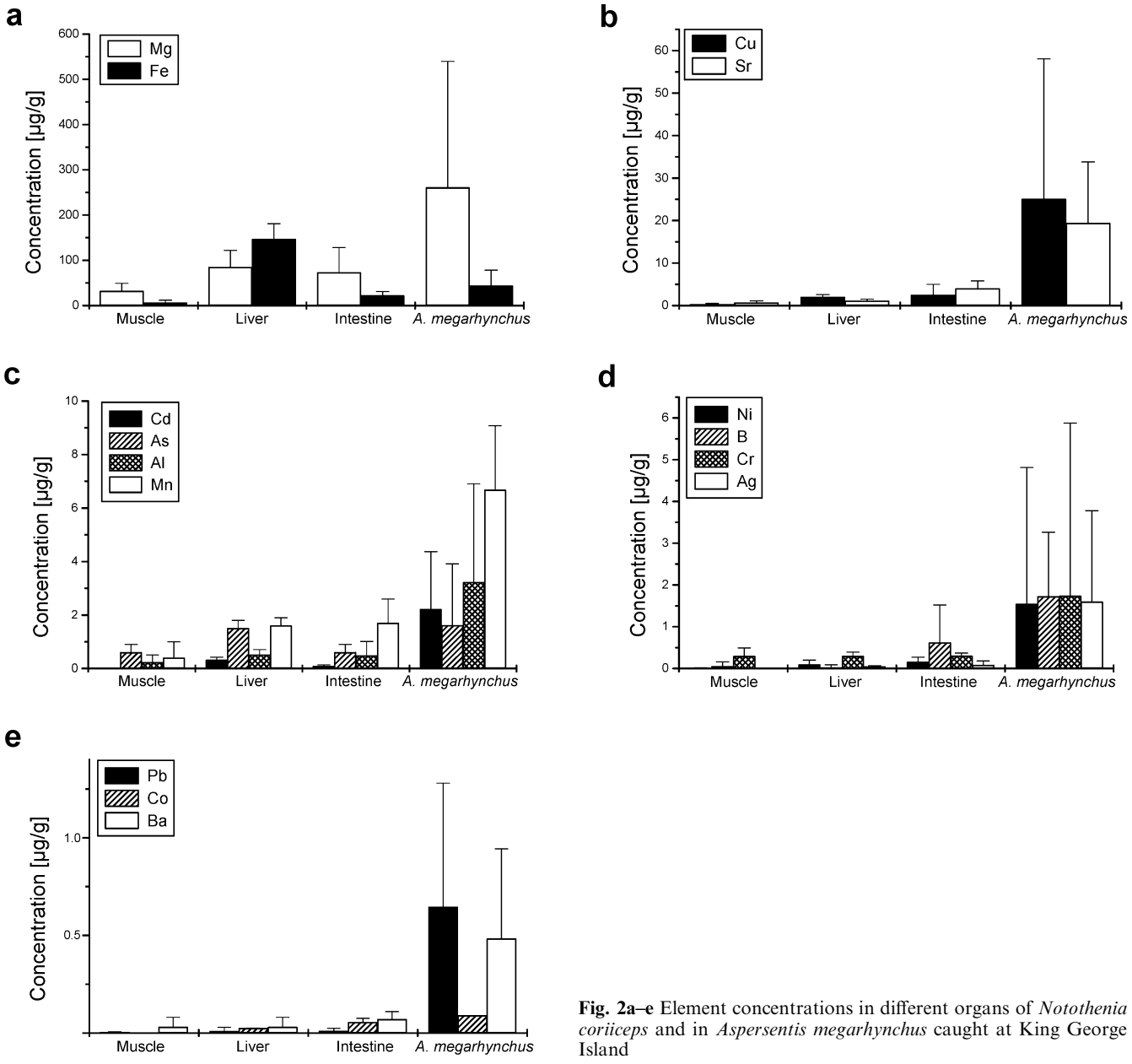

Fig. 2a–e Element concentrations in different organs of *Notothenia coriiceps* and in *Aspersentis megarhynchus* caught at King George Island

Element bioconcentration by *Aspersentis megarhynchus*

Bioconcentration factors (BCFs) were calculated as described earlier (Sures et al. 1999b) to compare the relative accumulation capacity of helminths with that of their host tissues (Table 3). The bioconcentration of elements in *Aspersentis megarhynchus* with respect to the intestinal wall of its host, listed in order of decreasing values, was as follows: Pb > Cd > Ag > Cu = Ni > Al = Ba > Cr > Sr > Mg = Mn > As = B > Fe = Co. Concerning the muscle of *N. coriiceps*, the maximum BCF was found for Cd (BCF = 2210), followed by Ni and Pb. Regarding the liver, B was the element that was bioconcentrated to the highest degree by the acanthocephalan, followed by Pb and Ag. The lowest BCF was 0.3 for the accumulation of Fe in *Aspersentis megarhynchus* compared with host's liver. Thus, the main metals in respect of their bioconcentration in the acanthocephalan were Pb, Cd, Ag, Ni and Cu.

Discussion

Most of the elements investigated in this study were accumulated to a higher degree in the acanthocephalan than in the tissues of its host. Only Fe was found in significantly higher concentrations in the liver of *N. coriiceps* than in the parasite. Fe is known to be an essential trace metal occurring, for example, in Fe-heme compounds like hemoglobin or myoglobin and also

Table 3 Accumulation factors calculated for the analysed elements in *Aspersentis megarhynchus* with respect to different organs of its host *Notothenia coriiceps*[a]

Element	Muscle	Liver	Intestine
Ag	n.d.[b]	36	20
Al	14	6	7
As	3	1	3
B	29	86	3
Ba	16	16	7
Cd	2210	7	24
Co	n.d.	4	2
Cr	6	6	6
Cu	81	15	10
Fe	7	0.3	2
Mg	8	3	4
Mn	18	4	4
Ni	375	17	10
Pb	325	72	65
Sr	29	17	5

[a] $C_{[\text{parasite}]}/C_{[\text{host organ}]}$, see Sures et al. (1999b).
[b] Not determined as the mean element level in host muscle was below the detection limit.

bound in proteins such as ferritin. Due to its important physiological role, this element is accumulated in the fish liver and in the reticuloendothelial cells as ferritin or hemosiderin (Huebers and Finch 1984). Higher Fe concentrations in fish liver compared with its acanthocephalans were also recently described for perch infected with *Acanthocephalus lucii* (Sures et al. 1999a), as well as for barbel infected with *Pomphorhynchus laevis* (Thielen et al. 2003). Furthermore, the Fe levels determined in the present study for *N. coriiceps* resembled data reported by Márquez et al. (1998) for the same fish species.

Additionally, from the data available on metal concentrations in *N. coriiceps*, it is clear that our results are similar to those mentioned in other reports although previous studies disregard metal concentrations in the intestine of *N. coriiceps* and in their parasites (de Moreno et al. 1997; Márquez et al. 1998; Vodopivez and Curtosi 1998). The element levels determined in *N. coriiceps* in the present study appear to be typical for marine fish when comparing our data with those from other studies (e.g. Hellou et al. 1992, 1996).

From the fact that element concentrations in the Antarctic fish species *N. coriiceps* are similar to those of marine fish caught in any other area of the world, it becomes obvious that toxic metals are present and thus available for the fauna of Antarctica, an ecosystem that is regarded as a pristine area that deserves protection. There is increasing concern that the recent growth of tourism and research activity may become a serious hazard for the Antarctic ecosystem (Sanchez-Hernandez 2000). Previous publications have stressed the need to develop and implement environmental monitoring programs, as well as environmental impact assessment procedures to conserve Antarctica and similarly evaluate the adverse effects of human activity (Abbott and Benninghoff 1990; Lyons 1993). Despite these needs, there is still a lack of studies on chemical and biological monitoring to determine natural trace-metal levels and to assess anthropogenic pollution in the Antarctic environment.

In recent years, some studies were performed using molluscs (e.g. Ahn et al. 1996; Lohan et al. 2001; Ahn et al. 2002; Duquesne and Riddle 2002) and crustaceans (Duquesne et al. 2000; Kahle and Zauke 2002) as accumulation indicators for different metals. Mussels such as *Laternula elliptica* appear to be among the most frequently used organisms for heavy-metal biomonitoring procedures in the Antarctic (e.g. Ahn et al. 1996; Lohan et al. 2001). The bivalve meets the criteria commonly accepted for sentinels in environmental monitoring, in terms of its abundance, and its accumulation capacity and tolerance against toxic effects of the pollutants (Beeby 2001). Comparison of the metal burdens in the mussel (Ahn et al. 1996; Lohan et al. 2001) with those in *Aspersentis megarhynchus* reveal that most elements determined in both organisms (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb) were accumulated to a higher degree in the acanthocephalan. Although the original data presented by Ahn et al. (1996) and Lohan et al. (2001) showed somewhat higher levels in the mussel as compared with the parasites, it must be considered that the mussel levels are given on a dry weight basis whereas metal burdens in *Aspersentis megarhynchus* were determined on a wet weight basis. Provided that the ratio dry weight:wet weight is 1:10, the parasites showed 8, 2.5, 2.5, 1.5, 1.5, 0.7 and 0.3 times higher concentrations for Cu, Cr, Mn, Pb, Ni, Fe and Cd, respectively, than the kidney of *L. elliptica* (Lohan et al. 2001). However, it would be interesting to directly compare metal concentrations in *L. elliptica* and *Aspersentis megarhynchus* sampled at the same time and at the same site, to verify if the levels are higher in the parasites.

Most of the elements were found in higher concentrations in *Aspersentis megarhynchus* than in the tissues of its host *N. coriiceps*. The value of acanthocephalans for the detection of metals becomes evident when comparing levels of Ag and Pb in the host tissues and in the parasites. The Pb levels in muscle, liver and intestine of the fish host were close to the detection limit, whereas the parasites accumulate this metal to a considerable level. Furthermore, Ag could not be detected in the muscle of *N. coriiceps* and the levels in liver and intestine were again close to the detection limit. In contrast, the parasites contained Ag levels that were approximately 36 times higher than in the liver. Thus, the question arises concerning the origin of silver pollution in the Antarctic. Recent studies on metals in the marine biota of Antarctica have not considered Ag but focussed on more common toxic metals like Cd, Pb and Hg (de Moreno et al. 1997; Sanchez-Hernandez 2000). The only record of Ag concentrations was for marine sediments sampled at the Antarctic Peninsula near Palmer and McMurdo stations (Kennicutt et al. 1995). Silver is regarded as a non-essential heavy metal used for jewelry, electroplating, and photographic and medicinal purposes (Saeki

et al. 2001). On a worldwide scale, this metal is discharged into the aquatic environment from domestic, agricultural, mining and industrial sources, with photoprocessing effluents as the main industrial pollution source (Wood et al. 1996). Due to its direct relation with human activities, increasing concentrations of this metal appear as a sensitive indication for anthropogenic influences in Antarctica. Photoprocessing and medical applications containing Ag are likely to be the main source of Ag pollution near research stations. From the study of Wood et al. (1996), it emerged that sediment-sampling sites near research stations showed a 3.5 times higher grade of silver pollution than the control site.

The use of extremely sensitive indicators, such as the acanthocephalan *Aspersentis megarhynchus*, aids the determination of the presence and availability of metals occurring only at low levels in the abiotic environment. In addition, other metals commonly associated with human activities in the Antarctic (e.g. Pb, Cd, Cu) were accumulated to a high degree in the parasites. Therefore, it is clear that acanthocephalans are not only capable of accumulating high amounts of toxic metals in freshwater habitats (e.g. Sures et al. 1999a) but also in the marine environment. Experimental studies on the question of whether the uptake and accumulation of metals in acanthocephalans is affected by the water characteristic, revealed that lead levels in *Paratenuisentis ambiguus* were independent of the salinity (freshwater—17‰) of the fish host's water (Zimmermann et al. 1999). However, a comparative study on the ability to concentrate metals in acanthocephalans, as compared with the host tissues from fish sampled in the marine environment, is still lacking. The only study that compared metal levels in parasites and their hosts investigated the accumulation of Pb and Cd in the pseudophyllidean cestode *Bothriocephalus scorpii* parasitizing turbot (Sures et al. 1997). In this study, however, the accumulation potential for metals in the parasites was low, with element concentrations being higher in the fish tissues than in the cestodes. In contrast, preliminary data on Pb in *Echinorhynchus gadi* suggested that acanthocephalans are probably able to bioconcentrate metals also in the marine environment (Sures et al. 1999b). Unfortunately, no metal concentrations for the host tissues were available. However, from the results presented here, it is clear that acanthocephalans parasitizing marine fish bioconcentrate a variety of elements to the same degree as acanthocephalans from freshwater fish such as *Acanthocephalus lucii* (Sures et al. 1999a) or *P. laevis* (Schludermann et al. 2003; Thielen et al. 2003).

In conclusion, it appears that acanthocephalans are very useful for assessing the presence and availability of metals in aquatic biotopes. Their enormous accumulation potential is advantageous for the determination of extremely low concentrations of pollutants which might, for example, occur in remote areas like the Antarctic.

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