Accumulation of heavy metals by intestinal helminths in fish: an overview and perspective

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SUMMARY

Intestinal helminths of fish are of increasing interest as potential bioindicators for heavy metal contamination in aquatic habitats. Among these parasites cestodes and acanthocephalans in particular have an enormous heavy metal accumulation capacity exceeding that of established free living sentinels. Metal concentrations several thousand times higher in acanthocephalans than in host tissues were described from field and laboratory studies. Whereas larval stages inside their intermediate hosts are not able to take up high quantities of metals, young worms begin to take up metals immediately after infection of the final host. After four to five weeks of exposure, the parasites reach a steady-state concentration orders of magnitude higher than the ambient water level. Thus, acanthocephalans are not only very effective in taking up metals, but they can also respond very rapidly to changes in environmental exposure. The mechanism which enable acanthocephalans to take up metals from the intestinal lumen of the host appears to be based on the presence of bile acids, which form organo-metallic complexes that are easily absorbed by the worms due to their lipophilicity. Investigations of the environmental conditions affecting metal uptake have shown that the parasites are more consistent and reliable indicators for metal pollution than the host tissues as metal levels of the latter are much more dependent on the water chemistry. Thus, after some years of research on the uptake of metals by acanthocephalans and on the factors affecting metal accumulation in intestinal parasites it should be asked if acanthocephalans meet the criteria commonly accepted for sentinels. If parasites can be considered as promising sentinels, we need reasons for the establishment of 'new' indicators. Therefore, this review summarises the present knowledge about parasites as bioindicators and compares the accumulation properties of parasites and established free living indicators. Finally, this review presents possible answers to the question why it could be advantageous to have new and even more sensitive indicators for environmental monitoring purposes.

Key words: Parasites, acanthocephalans, cestodes, bioindicators, pollution.

INTRODUCTION

In recent years, aquatic parasites have attracted increasing interest from an ecological viewpoint due to interactions with their hosts and their environment (Fig. 1). Among other factors, the viability and longevity of parasites are dependent on external environmental conditions. This direct impact of the environment on parasite longevity could be used in laboratory bioassays (see e.g. Morley, Crane & Lewis, 2001 *a*, *b*).

Aquatic hosts of parasites are also affected by environmental conditions. Pollution may adversely affect their health and even cause extinction. These detrimental changes are often associated with physiological reactions, which might be used as biomarkers (e.g. Segner, 1998; Segner & Braunbeck, 1998).

Host–parasite assemblages themselves are in the focus from a pathological point of view. Pathological studies of host–parasite interactions have led to numerous publications, describing the host–parasite interface from a morphological, immunological and biochemical point of view.

In addition to these interfaces, a more complex area deals with the coincidence of all parameters. From this interdisciplinary field different ways emerge which allow the indication of environmental pollution using host–parasite associations. The possible use of parasites as indicators for environmental quality has been reviewed recently (MacKenzie *et al.* 1995; Kennedy, 1997; Lafferty, 1997; Overstreet, 1997; Sures, Taraschewski & Siddall, 1997 *a*; Valtonen, Holmes & Koskivaara, 1997; Lafferty & Kuris, 1999; Sures, Siddall & Taraschewski, 1999 *a*; Sures, 2001). These reviews reveal that parasites have the potential to be used as effect indicators (Valtonen *et al.* 1997; Sures, 2001) and as accumulation indicators (Sures *et al.* 1999 *a*; Sures, 2001) as is known from free living animals (Gunkel, 1994).

In the case of effect indication with free living animals, changes in the physiology or behaviour of test organisms may be recorded in the presence or absence of environmental pollutants (Gunkel, 1994). One promising way to perform effect indication with parasites is the analysis of parasite population or community changes (e.g. Dušek, Gelnar & Šebelová, 1998; Valtonen *et al.* 1997) with respect to changes of environmental conditions. Although this procedure is time consuming, toxic effects were not only

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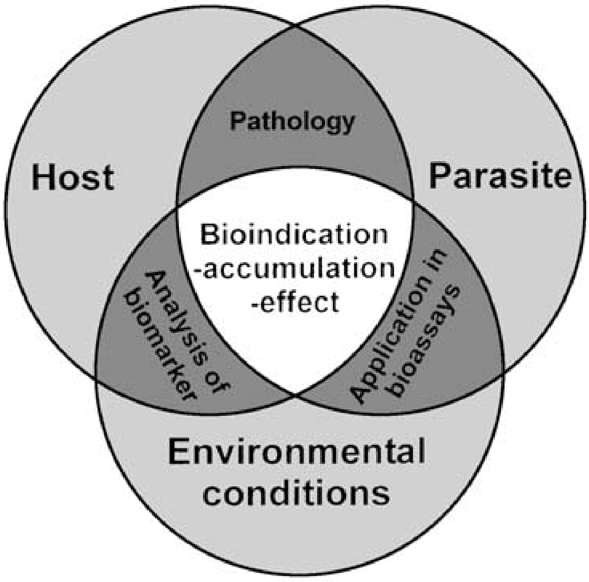

Fig. 1. Some interactions between the environment and host–parasite assemblages.

manifested by physiological changes in test animals but also by changes in population and community structure. Therefore, analysis of changes in the diversity and structure of parasite communities may be an integrative procedure to determine the integrity and health of ecosystems as it takes the life span of organisms into consideration.

On the other hand, bioindicators can be used as accumulation indicators. Such organisms provide valuable information about the chemical state of their environment through their ability to bioconcentrate substances of environmental concern within their tissues to levels surpassing the ambient concentrations. In recent years it has been shown that not only free living organisms like mussels and crustaceans (Dallinger, 1994) but also parasites are able to accumulate heavy metals within their tissues to values orders of magnitude higher than those in the host organs or the environment (Sures, 2001). The value of parasites as accumulation indicators for heavy metals in environmental monitoring will be discussed in the present review and thoroughly compared with that of established free living sentinels.

HEAVY METAL ACCUMULATION IN PARASITES

Until now, only endoparasites have been used in bioaccumulation studies. Most of the research has been conducted on endohelminths such as trematodes, cestodes, nematodes and acanthocephalans as their biomass is much greater than that of protozoans. Little information is available on metal accumulation in trematodes. There is one report dealing with Pb and Cd concentrations in the liver fluke *Fasciola hepatica* from cattle (Sures, Jürges & Taraschewski, 1998), but fish trematodes have not been investigated. Observations on metal levels in nematodes (Sures, Taraschewski & Jackwerth, 1994*a*; Sures *et al.* 1998; Szefer *et al.* 1998; Baruš *et al.* 1999*a*, *b*; Tenora *et al.* 1999*a*, *b*, 2000) indicate that these helminths are unsuitable as accumulation indicators since metals are present at low concentrations. In contrast, cestodes appear to be more promising metal accumulation indicators (Riggs, Lemly & Esch, 1987; Turčeková & Hanzelová, 1996; Sures, Taraschewski & Rokicki, 1997*b*; Tenora *et al.* 1997; Baruš, Tenora & Kráčmar, 2000*a*; Baruš *et al.* 2000*b*; Tenora *et al.* 2000; Sures, Grube & Taraschewski, 2002*a*), but more experimental studies are required to evaluate the reliability of tapeworms as sentinels. Acanthocephalans are probably the best investigated helminths as a result of their excellent metal accumulation capacities (see e.g. Sures *et al.* 1999*a*; Sures, 2001). There is not only a good collection of field data (Sures *et al.* 1994*a*, *b*, *c*; Sures & Taraschewski, 1995; Sures, Taraschewski & Rydlo, 1997*c*; Sures *et al.* 1999*b*; Sures, Franken & Taraschewski, 2000*a*; Sures, 2002*a*, *b*; Sures & Reimann, 2003), but there are also some results based on laboratory investigations (Siddall & Sures, 1998; Sures & Siddall, 1999; Zimmermann, Sures & Taraschewski, 1999; Scheef, Sures & Taraschewski, 2000; Sures, Jürges & Tarschewski, 2000*b*; Sures & Siddall, 2001, 2003). Due to the need for sentinel species in terrestrial, especially urban, habitats (Beeby, 2001) an increasing number of papers deal with metals in helminths parasitising mammals, although most of the studies cited above focus on metal bioconcentration in parasites from fish. It is easier to undertake experimental studies on the uptake and accumulation of metals by parasites of aquatic animals as they take up metals predominantly via the water rather than food. Therefore, it will be possible to compare the rate of metal accumulation and elimination by different fish-acanthocephalan combinations as well as the relationship between the exposure and steady-state tissue concentration. After having evaluated these relationships it will be possible to adopt the technology to terrestrial host parasite assemblages.

METAL ACCUMULATION IN FISH ACANTHOCEPHALANS

The first study on metal uptake by acanthocephalans was concerned with Cd accumulation by cystacanths of *Pomphorhynchus laevis* dissected from experimentally exposed, naturally infected *Gammarus pulex* (Brown & Pascoe, 1989). However, in this study these larvae had concentrations of Cd lower than in the intermediate hosts. Additional studies on metal levels in different larval acanthocephalan species revealed that only adult worms located in the intestine of the definitive host are able to accumulate metals (Sures & Taraschewski, 1995; Siddall & Sures, 1998; Sures & Siddall, 2001). Comparison of

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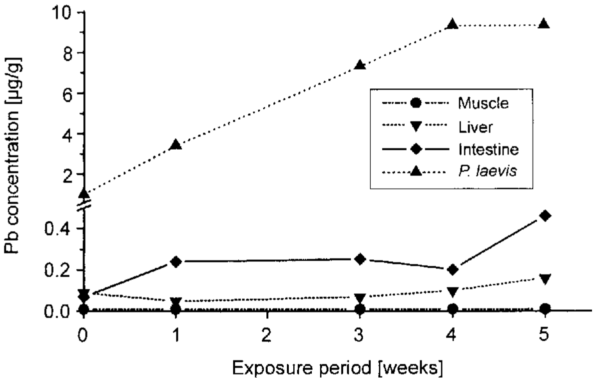

Fig. 2. Uptake of lead by chub (*Leuciscus cephalus*) experimentally infected with *Pomphorhynchus laevis* (data from Sures & Siddall, 2003).

lead and cadmium concentrations in larval and adult *Acanthocephalus lucii* with those in their intermediate and definitive hosts (all animals sampled at the same site) revealed that the adults contained 370 times higher lead levels than the muscle of perch and 30 times more lead than the larvae (Sures *et al.* 1994*c*; Sures & Taraschewski, 1995). A similar picture emerged for cadmium with levels in the adults being 120 times higher than in the muscle of perch and 180 times higher than in larvae dissected from *Asellus aquaticus* (Sures *et al.* 1994*c*; Sures & Taraschewski, 1995). Also other common fish species such as chub (*Leuciscus cephalus*) and eel (*Anguilla anguilla*), both infected with acanthocephalans, have been sampled from moderately polluted sites in Germany. Again, the concentrations of lead and cadmium in the parasites were orders of magnitude higher than in the host tissues (muscle, liver and intestine) or the aquatic environment. For example, mean concentrations of lead and cadmium in *Pomphorhynchus laevis* were, respectively, 2700 and 400 times higher than the muscle of the host (*L. cephalus*) and 11 000 and 27 000 times higher than in the water column (Sures *et al.* 1994*b*; Sures & Taraschewski, 1995).

Following these field studies, laboratory experiments were conducted to study the time-course of lead accumulation in different fish tissues and the acanthocephalans over five weeks of exposure to an aqueous lead concentration of 0·01 mg l^{-1} (Sures & Siddall, 2003). Lead was accumulated rapidly in intestinal worms reaching a steady-state concentration after 4 weeks, which was 9000 times higher than the exposure concentration (Fig. 2). There were marked differences in the accumulation kinetics for lead in the parasite and the tissues of chub. After a 4–5 weeks exposure to lead the levels of this metal continued to increase significantly in the liver and intestine of chub but not in the muscle. After 5 weeks of exposure the parasites contained 20, 58 and 930 times more lead than the intestine, liver and muscle of their host, respectively.

Additional experiments showed that the mass of lead accumulating in *P. laevis* was positively correlated with the exposure concentration but there was no relationship with either parasite intensity or with pooled or individual worm weight. The lead concentrations were higher in specimens of *P. laevis* attached in the posterior part of the intestine than in more anteriorly located worms (Sures & Siddall, 2003). Furthermore, the high metal accumulation capacity of *P. laevis* results in a reduction in the amount of lead accumulating in the intestinal wall but not in the amount accumulating in the liver of chub (Sures & Siddall, 1999).

Parasites and fish hosts may compete for several other elements as well as for lead (Sures, 2002*a*), demonstrating that concentrations of metals such as calcium, iron, zinc and strontium in the liver of perch were negatively correlated with the size of *Acanthocephalus lucii* infrapopulations. Competition of essential metals by *A. lucii* may explain parasite-induced skeleton deformation of final hosts such as shortened gill opercula and deformation of the spinal cord of trout (Taraschewski, 2000), heavily infected by acanthocephalans. Although similar symptoms in fish have also been described in association with other infectious agents (Amlacher, 1992) and even with heavy metal intoxication (Spry & Wiener, 1991) the uptake of minerals by acanthocephalans might be involved in skeletal deformations.

In addition to competition for elements between the host and its parasites there is also competition among essential elements (Ba, Ca, Fe, Mn, Sr and Zn) between individual acanthocephalans inside the gut of the host (Sures, 2002*a*). As these metals are of physiological importance to most animals (Merian, 1991), it is conceivable that competition between parasites for these may lead to an increased absorption of other, non-essential or even toxic elements such as lead and cadmium. This idea is supported by experimental data on lead accumulation in the acanthocephalan *Paratenuisentis ambiguus* and its final host *Anguilla anguilla*. Following exposure of the host–parasite-system to Pb depending on the water hardness (Sures *et al.*, unpublished), decreased Pb levels were found with increased Ca concentrations of the water.

The accumulation of metals also occurs in acanthocephalan parasites of marine fish like *Gadus morrhua* (Sures, 2001) and *Notothenia coriiceps* (Sures, 2002*b*; Sures & Reimann, 2003). Experimental studies on the impact of the water chemistry on lead accumulation in the host parasite system eel (*A. anguilla*) and *Paratenuisentis ambiguus* showed that salinity reduces the lead burden in host tissues whereas it has no effect on the lead accumulation in the acanthocephalans (Zimmermann *et al.* 1999). Furthermore, although the lead uptake by fish is still a controversial issue (Spry & Wiener, 1991), water-borne and trophic exposures of eels to lead cause similar lead

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concentrations in the parasites whereas the mode of lead application significantly affects metal levels in eel tissues. Therefore, acanthocephalans appear to be promising biological indicators for water quality, not only in freshwater, but also in marine and estuarine ecosystems regardless of the pollution source.

MECHANISM OF METAL UPTAKE

Although cystacanths inside their intermediate hosts are not able to take up metals (see previous paragraph), young acanthocephalans only four days post-infection of the definitive host were able to bioconcentrate metals to a very high degree (Siddall & Sures, 1998). The chemistry of the gut microhabitat is the decisive factor determining the accumulation of metals by acanthocephalans (Sures & Siddall, 1999, 2001). Bile acids facilitate the uptake of metals by forming organo-metallic complexes which are more bioavailable than ionic metals (Sures & Siddall, 1999; Zimmermann *et al*. 2002*a*). As acanthocephalans cannot synthesise their own cholesterol and fatty acids they have become proficient in sequestering them from the host's intestinal lumen. The production of bile by the host is therefore extremely important in the development of acanthocephalans. Recent *in vitro* studies demonstrated that bile salts are necessary to activate the larval cystacanths and enhance the metal uptake of acanthocephalans (Sures & Siddall, 1999). After three weeks maintenance of *P. laevis* cystacanths in medium (RPMI 1640) containing bile acids the mean individual weight of the worms increased threefold compared with cystacanths which were maintained in medium without bile. This activation of the bile-treated acanthocephalans was also manifested by an eversion of the proboscis. Comparison of lead uptake by these larvae revealed that lead levels in acanthocephalans exposed to Pb in medium nearly doubled when 1% bile was added, from an approximate mean concentration of 11 $\mu g\ g^{-1}$ wet weight (bile absent) to 18 $\mu g\ g^{-1}$ wet weight (bile present, see Sures & Siddall, 1999).

In conclusion, the absorption of bile-bound lead by *P. laevis* in the host's intestine benefits the fish host by reducing the reabsorption of the metal by the intestinal wall (Sures & Siddall, 1999).

HEAVY METAL ACCUMULATION IN ACANTHOCEPHALANS COMPARED WITH ESTABLISHED BIOINDICATORS

Before parasites can be established as accumulation indicators for heavy metals it is necessary to compare their accumulation capacity with that of established free living organisms such as the zebra mussel *Dreissena polymorpha* (Cope *et al*. 1999; Roditi & Fisher, 1999; Sures *et al*. 1999*b*; Roditi, Fisher & Sanudo-Wilhelmy, 2000; Zimmermann *et al*. 2002*b*).

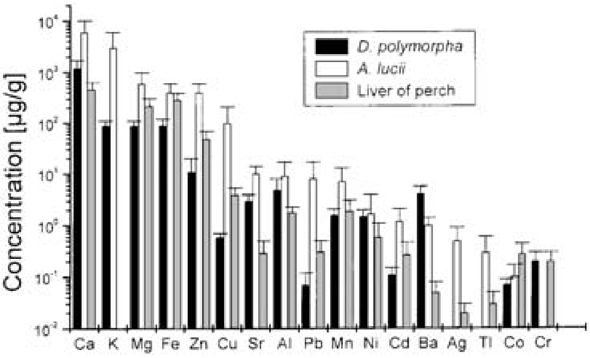

Fig. 3. Element concentrations (mean ± S.D.) in the liver of perch, its intestinal parasite *Acanthocephalus lucii* and in *Dreissena polymorpha*, sampled at the same site in lake Mondsee, Austria (data from Sures *et al*. 1999*b*).

Accordingly, studies were carried out to determine metal concentrations in *Acanthocephalus lucii* from naturally infected perch, *Perca fluviatilis*, and in zebra mussels from the same site (Sures *et al*. 1997*c*, 1999*b*). The comparison of lead and cadmium concentrations in these organisms sampled at a site which receive effluent from a highway compared to a reference site, not obviously affected by traffic related pollution, demonstrate that zebra mussels are more suitable to detect localized differences in contamination than fish or their endoparasites (Sures *et al*. 1997*c*). The ability to discriminate between gradients of pollution is most likely due to the immobility of the mussel which is attached to the substratum by byssal threads. In contrast to mussels, perch and their acanthocephalans are more mobile and thus less precise indicators of localized differences in pollution. However, the bioconcentration of lead and cadmium in *A. lucii* was several times greater than that in zebra mussels (Sures *et al*. 1997*c*). Furthermore, not only lead and cadmium were bioconcentrated in the parasites to a higher degree than in the mussels, but also several other metals (see Fig. 3). Except for barium and chromium, all other element concentrations were significantly lower in *D. polymorpha* than in *A. lucii* and all except cobalt, iron and chromium were accumulated above fish host liver levels in the parasites (for details see Sures *et al*. 1999*b*). However, there is a higher degree of variability among the metal burden of the parasites than among individual mussels. This variability, which may reflect the mobility of the fish host, can obscure the differences that might otherwise be detected between sites. Despite this, acanthocephalans can provide ecologically valuable information on the average exposure of a mobile fish host within its natural home range. For active monitoring purposes infected fish could be exposed in weir-baskets at different locations to eliminate problems associated with the fish host's mobility. Presently there is an ongoing field study comparing metal concentrations in water and sediment of the river Danube with

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Table 1. Characteristics of ideal sentinal species (according to Martin & Coughtrey, 1982; Phillips & Segar, 1986; Phillips & Rainbow, 1993; Beeby, 2001)

Criteria	Acanthocephala
Rapid equilibration with source	Yes
A linear relationship with source over the range of ambient concentrations	Yes[1]
The relationship between the tissue and source concentrations should be the same at all sites studied	[1]
Abundant species from which large numbers can be taken without altering the age structure or having some other significant effect on the population	Yes
Easily identified	Yes
Large body of knowledge about the species' physiology, including the effects of age, size, season and reproductive activity on the assimilation of the pollutant	No[1]
Large body – to provide abundant tissue for analysis	Yes
Sedentary or with a well defined home range	Yes[2]
Uptake is from a well defined pollution source	Yes
Easily aged and long lived – allowing integration of the pollutant over long periods	[3]

[1] More information required.
[2] The home range of a parasite species corresponds to that of its host.
[3] See discussion in the text.

those in barbel and its acanthocephalan parasites, as well as in *D. polymorpha* (Schludermann *et al.* 2003).

The high accumulation potential for several metals suggests that acanthocephalans are even more useful as environmental indicators for assessing metal pollution in aquatic habitats than established indicator organisms. Therefore, it is necessary to evaluate if acanthocephalans meet the criteria commonly accepted for sentinels. The most important characteristics for ideal sentinel species (according to Martin & Coughtrey, 1982; Phillips & Segar, 1986; Phillips & Rainbow, 1993; Beeby, 2001) are listed in Table 1. Although the idea to use parasites as sentinels is comparatively new, we know from recent studies that acanthocephalans meet most of the criteria for ideal sentinels (see Table 1). However, there are some characteristics where we do not have enough data to decide whether parasites fulfil the necessary criteria. But we should see this mainly as a request to intensify research on parasites as indicators.

The only demand which could not be addressed for acanthocephalans is that they should be easily aged and long lived. An age determination of acanthocephalans appears to be impossible. But as the estimated life-time of acanthocephalans ranges from 50 to 140 days (Dobson & Keymer, 1985; Kennedy, 1985), it seems unnecessary to specify the exact age. Due to the outer appearance it is possible to decide whether the worms are very young or fully mature.

Furthermore, as suggested by Sures *et al.* (1999*a*) the most informative way to perform accumulation indication with acanthocephalans may be the use of a two component model, which comprises the parasite and its host. Following metal analysis in the parasites and in selected host tissues like e.g. liver and muscle, ratios of the metal concentration in the parasites to those in the host tissue ($C_{[\text{parasite}]}/C_{[\text{host tissue}]}$) can be determined. For example, the ratio between metal concentrations of the parasites and host muscle tissue could provide information on the duration of environmental exposure as metal uptake occurs more rapidly in the parasites. Thus, after short-term exposure a high ratio could be expected as the parasite would have accumulated much more of the contaminant than the muscle of the host (see e.g. Sures & Siddall, 2003). Relatively high metal levels in both host muscle and parasites (i.e. low ratio) would indicate a longer exposure time. By combining the information which derives from the host and its parasites in the form of such a ratio, an estimation of the concentration and duration of the exposure may be possible which could not be achieved by using only one indicator species like the zebra mussel. Additionally, in respect of an integration of the pollutant over long periods of time the use of host-parasites assemblages is a combination of a short-term indicator (acanthocephalan, some days to months) and a long-term indicator (fish host, up to some years). These combined results seem very useful for environmental monitoring purposes. However, we are just at the beginning to recognize these advantages associated with the use of parasites and their hosts as accumulation indicators and therefore need more studies aiming at the applicability and reliability of this system as sentinel.

WHY DO WE NEED 'NEW' ACCUMULATION INDICATORS?

Although there are good arguments to establish new organisms as sentinels, ecologists prefer to use animals like mussels, crustaceans and fish for indication purposes (Rosenberg & Resh, 1993). On the one hand, they are familiar with these organisms and on the other hand one may ask why we should need new accumulation indicators. This question is especially important as we are dealing with endoparasites of vertebrates. That means, it is necessary to kill and dissect a vertebrate host before the parasites can be isolated. But the need to monitor the heavy metal contents in fish and other aquatic animals taken for human consumption is generally accepted from a public health viewpoint. Therefore, after dissecting

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fish, their parasites can be easily removed from the intestine. But instead of considering parasites like acanthocephalans as indicators while routinely analysing the metal contents of edible parts of fish, free living invertebrates such as mussels or crustaceans, which have even lower metal accumulation rates than adult acanthocephalans, are commonly sampled from ecosystems and analysed for their metal burden.

Additionally, by using acanthocephalans in environmental impact studies, even the lowest metal concentrations can be detected in the water due to the enormous accumulation capacity of these worms. Therefore, the use of parasites might be helpful for the exploration of remote areas, like e.g. the Antarctic (see Sures, 2002*b*; Sures & Reimann, 2003) to investigate whether a specific metal is present in a given habitat at bioavailable concentrations or not. In addition to remote areas where concentrations of metals are rather low (Sanchez-Hernandez, 2000), a new man-made metal pollution justifies the use of the most efficient accumulation indicators. This pollution is caused by cars which are equipped with catalytic converters. Following the introduction of automotive catalytic converters the platinum group elements (PGE) palladium (Pd), platinum (Pt) and rhodium (Rh) are emitted with exhaust fumes (Palacios *et al*. 2000). These noble metals are used as active components in converters for reducing the emission of hydrocarbons, carbon monoxide and nitrogen oxides. Although field studies demonstrate a cumulative increase of PGE concentrations in road dust and soils along heavily frequented roads (reviewed in Hoppstock & Sures, 2003), there is presently only a poor dataset on the biological availability of these metals (Sures *et al*. 2001, 2002*b*, 2003; Zimmermann *et al*. 2002*b*). In a recent study on European eels naturally infected with the eoacanthocephalan parasite, *Paratenuisentis ambiguus*, which were experimentally exposed to ground catalytic converter material, the parasites take up and accumulate Pt and Rh whereas in the host tissues examined no metal uptake was detected (Sures *et al*. 2003). Compared to the concentrations in the water the worms contained 1600 times higher Rh and 50 times higher Pt concentrations. This study showed that acanthocephalans can be used to indicate whether a specific metal is present in a given habitat or not. Furthermore, due to their enormous accumulation capacity such effective bioindicators may enable the chemical proof of pollutants by a preconcentration of metals inside their body prior to metal analysis.

The studies reviewed here provide good evidence that acanthocephalans and their hosts are promising indicators for metal pollution. Very high metal levels were not only described for acanthocephalans in aquatic habitats but also for parasites from terrestrial hosts. Therefore, it is worth intensifying the research in this field.

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