Bioaccumulation of metals by the southern mouthbrooder, *Pseudocrenilabrus philander* (Weber, 1897) from a mine-polluted impoundment

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Abstract

The bioaccumulation of Fe, Mn, Zn, Cu, Ni and Pb by the cichlid *Pseudocrenilabrus philander* from a mine-polluted impoundment in the Transvaal was investigated. With the exception of Fe, all the other metals were accumulated in higher concentrations in the tissues of the fish than those in the sediments of the lake, with the highest bioconcentration factor being 8.54 for Zn. Results also showed that there was an inverse relationship between metal concentration and body mass of the fish, with the smaller juvenile fish being better able to concentrate all the metals per equivalent body mass than was the case for the larger, adult fish. This phenomenon is linked to a superior bioregulation mechanism for metals by the larger older fish, as well as the relatively higher metabolic rate of the younger juvenile fish.

Introduction

The mining of gold-bearing ores over decades on the Witwatersrand and the deposition of waste products above ground has resulted in the continuous leaching of minerals from these dumps into streams, lakes and rivers. These effluents contributed significantly towards the acidification, mineralisation and metal contamination of the affected water bodies (Wittmann and Förstner, 1976a; 1976b). Changes in the pH of the water have a direct bearing on the solubility and deposition of such metals in the bottom sediments of standing and flowing water ecosystems (Förstner and Prosi, 1979). Plants (Whitton et al., 1981; Van der Merwe et al., 1990) and benthic macro-invertebrates (Nehring, 1976; Eyrest and Pugh-Thomas, 1978) are thus able to utilise these metals directly or indirectly from the sediments. Such metals are transferred from the plants and macro-invertebrate fauna to freshwater fish via several pathways in the food chains in the affected ecosystems (Heath, 1987). Fish which are present in such waters may obtain these metals by means of diffusion through gill and skin surfaces (Matthiessen and Brafield, 1977; Heath, 1987) or, from their natural food (Mathis and Cummings, 1973; Moore and Ramamoorthy, 1984; Villegas-Navarro and Villarreal-Trevino, 1989).

Conflicting reports exist concerning the mechanism involved, and/or the ability of fish to bioconcentrate the various metals in their organs and tissues. Some researchers have found that a positive correlation exists between fish body mass and metal concentration (Phillips et al., 1980; Mohamed et al., 1990), whilst others (Goodyear and Boyd, 1972; Johnson, 1987) recorded no correlation between these two parameters. In some cases, however (Chernoff and Dooley, 1979; Anderson and Spear, 1980; Memmert, 1987), a definite inverse relationship was found to exist between body mass and metal concentration.

In the case of the mine-polluted Spaarwater Pan (Fig. 1), a number of fish species still occur, including the cichlid, *Pseudocrenilabrus philander*, also known as the southern mouthbrooder, which is mainly confined to the shallow littoral

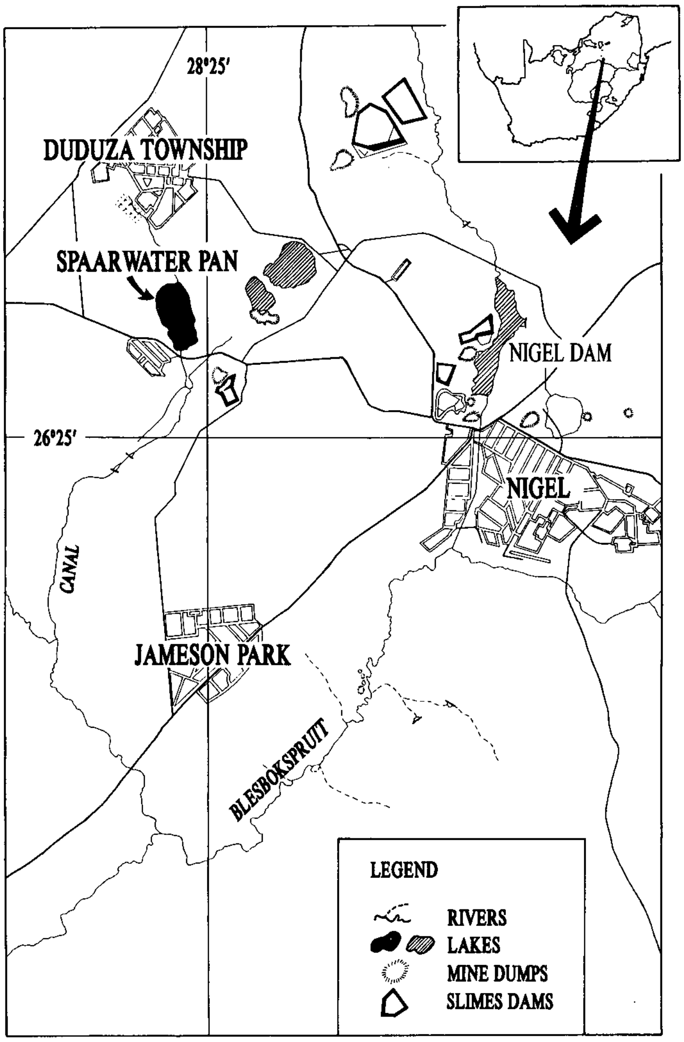

Figure 1
Map showing the location of the Spaarwater Pan near Nigel

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zone of the lake. This fish is known to feed actively on benthic macro-invertebrates, including freshwater crustaceans and insect larvae as well as small fish (Polling et al., 1994; Schoonbee, personal observations). A certain amount of detritus is also found in the diet of this fish, reflecting its benthic feeding habits.

In the present study, investigations were made into the ability of *P. philander* to bioaccumulate the metals Fe, Mn, Zn, Cu, Ni and Pb from the aquatic environment of this impoundment which contains appreciable quantities of the metals mentioned in both the sediments and the water. Investigations were also made to determine the ability of its smaller juveniles to accumulate these metals in higher concentrations per equivalent mass than the larger older fish.

Materials and methods

Collection and preparation of samples

A modified Moore shocker (Moore, 1968) was used to collect 389 individuals of *P. philander* at 4 localities in the littoral zone of the Spaarwater Pan. Water and sediment samples were also taken at these sites for various physical and chemical analyses, including metal analyses (*Standard Methods*, 1989). Fish were individually weighed and the total and standard lengths determined. All sediment and fish samples were dried individually in an oven at 90°C for a minimum period of 48 h, and the dry mass determined. Due to the relative smallness of the juveniles, specimens below 0.5 g individual wet mass were pooled in certain successive mass groups to allow for sufficient material in each case for AAS determination of the six metals. The smallest specimens (47 in total), ranging from 0.0200 g to 0.1799 g, were grouped in successive size classes with intervals of 0.04 g. This was followed by mass groups of juveniles ranging from 0.1800 to 0.2799 g (30 specimens) which were pooled for successive 0.02 g mass intervals. The largest of the juvenile fish, ranging between 0.2500 and 0.4999 g in weight, were combined in successive groups of 0.01 g intervals. Fish larger than 0.5 g wet mass were large enough to be analysed individually following the drying process. A total of 264 fish samples, which included the composite (smaller) and the individual (larger) fish were thus prepared for whole body AAS analysis.

All dried samples, including the collected water, were digested separately according to standard procedures (Van Loon, 1980) using a 1:1 perchloric and nitric acid mixture. The period of digestion lasted for at least 4 h, during which time total digestion and clearing of the samples occurred. Each digested sample was then filtered separately using a 6 µm Millipore filter paper. Individually filtered samples were then made up to 100 mℓ with double distilled deionised, metal-free water and transferred to separate clean metal-free and labelled storage bottles.

Atomic absorption analysis of samples

A Varian SpectrAA-10 Atomic Absorption Spectrophotometer was used to determine the concentration of Fe, Zn, Mn, Cu, Ni and Pb in the water, sediments and fish samples. The results obtained were recalculated to µg/g using the actual dry mass of each digested sediment and fish samples. In the case of the water, results are expressed in µg/ℓ. Bioconcentration ratios (BCRs) (Heath, 1987; Sorensen, 1991) were calculated for all the fish samples using the following equation:

$$BCR = [M]_f / [M]_s$$

where:
[M] = metal concentration
f and s refer to the fish and sediments, respectively.

Results were statistically evaluated using Spearman's correlation coefficient and a t-test on a 99 % scale of significance (Zar, 1984). For the t-test a t-value was calculated using the following equation:

$$t = r \sqrt{n-2} / \sqrt{1-r^2}$$

where:
r = correlation coefficient
n = size of sample.

The degrees of freedom (df) were 262. Data were illustrated graphically using Harvard Graphics.

TABLE 1
PHYSICAL AND CHEMICAL CONDITIONS IN THE SPAARWATER PAN IN SUMMER (NOVEMBER, 1992) AT THE TIME OF THE SURVEY (n = 4)

Water quality parameters	$\bar{x}$	Range
pH	7.9	7.6 - 8.1
Conductivity (µS/cm)	1038	850 - 1300
Dissolved oxygen (mg/ℓ)	3.9	1.4 - 6.2
Temperature (°C)	22.5	22 - 23
Alkalinity (mg/ℓ $CaCO_3$)	196	168 - 228
Hardness (mg/ℓ $CaCO_3$)	179	159 - 191
Ammonia nitrogen (mg/ℓ NH_3)	0.3	0.2 - 0.4
Nitrite nitrogen (mg/ℓ NO_2)	0.01	-
Nitrate nitrogen (mg/ℓ NO_3)	0.8	0.6 - 1.2
Orthophosphate (mg/ℓ PO_4)	0.06	0.04 - 0.07
Sulphate (mg/ℓ SO_4)	175	150 - 200
Turbidity (NTU)	< 5	-

TABLE 2
METAL CONCENTRATIONS IN THE WATER, SEDIMENTS AND IN *P. PHILANDER,* AS WELL AS THE BIOCONCENTRATION RATIOS (BCRs) FOR THE VARIOUS METALS IN THE FISH AND SEDIMENTS IN THE SPAARWATER PAN. RESULTS ARE BASED ON DRY MASS VALUES OF THE SEDIMENTS AND THE WHOLE BODY DRY MASS ANALYSIS OF THE FISH

		Metals					
Parameters		**Fe**	**Mn**	**Zn**	**Cu**	**Ni**	**Pb**
Water	x̄	1 119	88	34	7	37	48
(μg/ℓ)	SD	1 764	47	17	4	11	5
n = 4	CV %	109.0	53.0	49.6	52.5	29.4	11.2
	Range	263 - 4650	53 - 169	20 - 63	5 - 13	28 - 56	43 - 56
Sediments	x̄	5 979	76	33	7	41	36
(μg/g)	SD	5 984.0	20.8	11.3	4.2	7.7	5.8
n = 4	CV %	100.1	27.2	34.6	57.0	18.5	15.9
	Range	1 720 - 16 210	49 - 97	21 - 51	4 - 14	34 - 54	29 - 44
Fish	x̄	595	99	279	9	80	71
(μg/g)	SD	329.0	34.6	66.8	3.1	47.0	35.6
n = 264	CV %	55.0	35.1	23.9	34.8	59.1	50.0
	Range	105 - 3 246	41 - 291	154 - 549	4 - 26	7 - 333	10 - 162
Concentration	x̄	0.10	1.29	8.54	1.23	1.92	1.96
Ratio (BCR):	SD	0.06	0.45	2.04	0.43	1.13	0.98
Fish/sediment	CV %	55.0	35.1	23.9	34.8	59.1	50.0
ratio (n=264)	Range	0.02 - 0.54	0.54 - 3.83	4.67 - 16.64	0.57 - 3.71	0.17 - 8.12	0.28 - 4.50

Results

Results on the water chemistry showed the water of the impoundment to be alkaline, with a pH fluctuating between 7.6 and 8.1 and with a mean calculated pH of 7.9 (Table 1). The summer water temperature measured exceeded 22°C in all cases. The alkaline nature of the water was further demonstrated by values obtained for alkalinity and hardness. Conductivity values ranged between 850 μS/cm and 1 300 μS/cm, with a mean of 1 038 μS/cm. This, together with the sulphate values, which fluctuated between 150 mg/ℓ and 200 mg/ℓ, clearly reflects the effect of waste-water seepage from the surrounding mine dumps into the dam. Ammonia, nitrite, nitrate and orthophosphate suggest some degree of organic contamination of the water (Table 1). The turbidity of the water is low, less than 5 NTU.

The wet mass of all individuals of *P. philander* collected in the Spaarwater Pan ranged between 0.048 g and 9.534 g and the length between 17.5 mm total length (14.5 mm standard length) and 81 mm total length (66 mm standard length). A mean moisture content of 78.5 % was obtained for all fish specimens.

The mean metal content in the water, sediments and fish, and the fish/sediment bioconcentration ratios (BCRs), are summarised in Table 2. Of the 6 metals, iron occurred in the highest concentrations in both the water (μg/ℓ) and sediments (μg/g). Manganese (88 μg/ℓ) showed the second highest concentration of all 6 metals in the water, followed by Pb (48 μg/ℓ), Ni (37 μg/ℓ), Zn (34 μg/ℓ) and Cu (7 μg/ℓ). In the case of the sediments, Mn with a concentration of 76 μg/g dry mass, showed the second highest concentration of all the metals, followed by Ni (41 μg/g), Pb (36 μg/g), Zn (33 μg/g) and Cu (7 μg/g). With the exception of Zn, concentra-

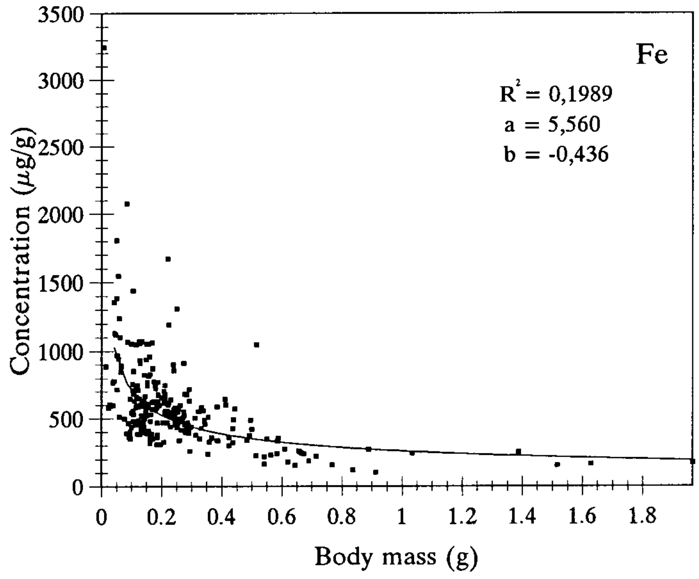

Figure 2
Relationship between iron concentration (μg/g) and body mass of P. philander *expressed in dry mass (g)*

TABLE 3
EVALUATION OF THE FISH MASS/METAL CONCENTRATIONS FOR SUCCESSIVE MASS GROUPS RANGING FROM 0.008 g TO 2.117 g OF *P. PHILANDER* IN THE SPAARWATER PAN

	Metals					
Statistics	**Fe**	**Mn**	**Zn**	**Cu**	**Ni**	**Pb**
df	> 100	> 100	> 100	> 100	> 100	> 100
r	-0.429	-0.401	-0.535	-0.573	-0.522	-0.615
t	-5.807	-5.487	-6.989	-7.400	-6.849	-7.834
Cv	-2.626	-2.626	-2.626	-2.626	-2.626	-2.626
P	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005

df = degrees of freedom = n - 2 = 262
r = correlation coefficient
t = calculated t-value
Cv = critical value
P = probability on the 99% level of confidence

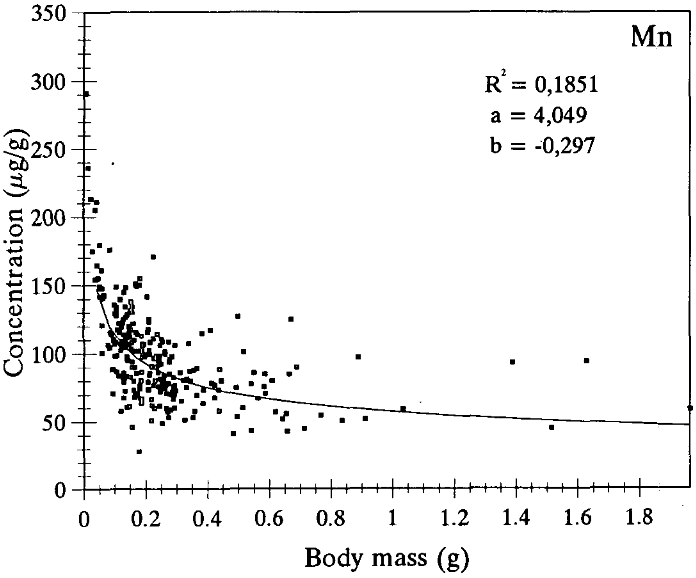

Figure 3
Relationship between manganese concentration (µg/g) and body mass of P. philander *expressed in dry mass (g)*

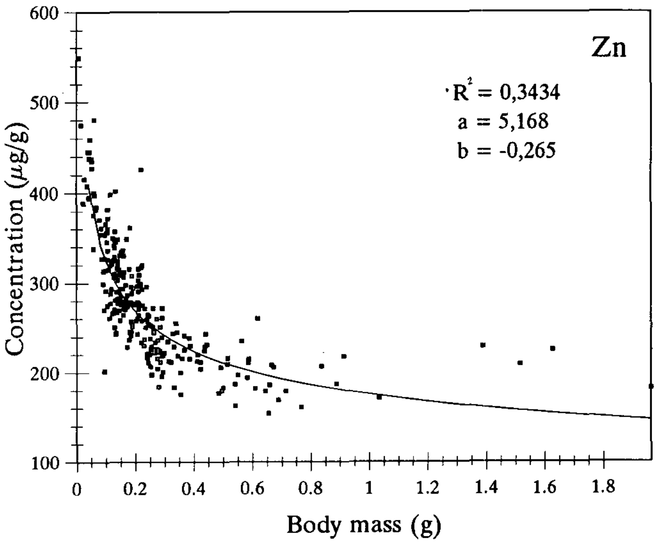

Figure 4
Relationship between zinc concentration (µg/g) and body mass of P. philander *expressed in dry mass (g)*

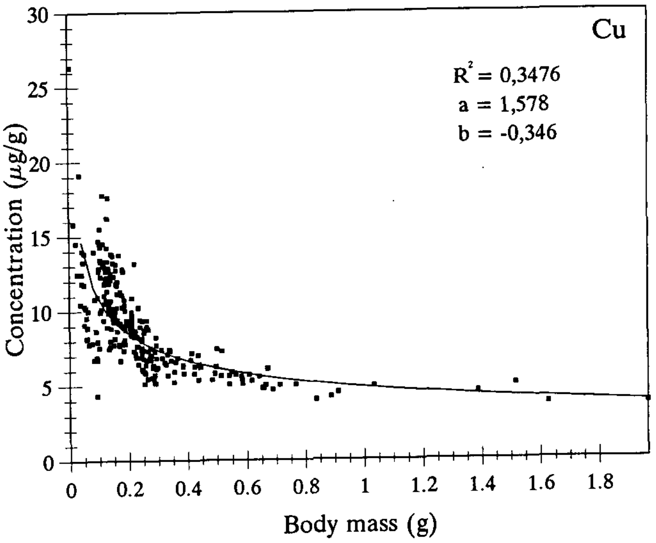

Figure 5
Relationship between copper concentration (µg/g) and body mass of P. philander *expressed in dry mass (g)*

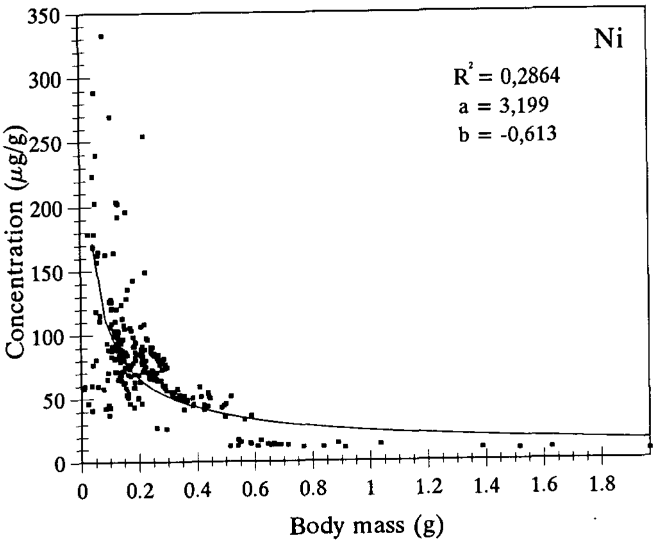

Figure 6
Relationship between nickel concentration (µg/g) and body mass of P. philander *expressed in dry mass (g)*

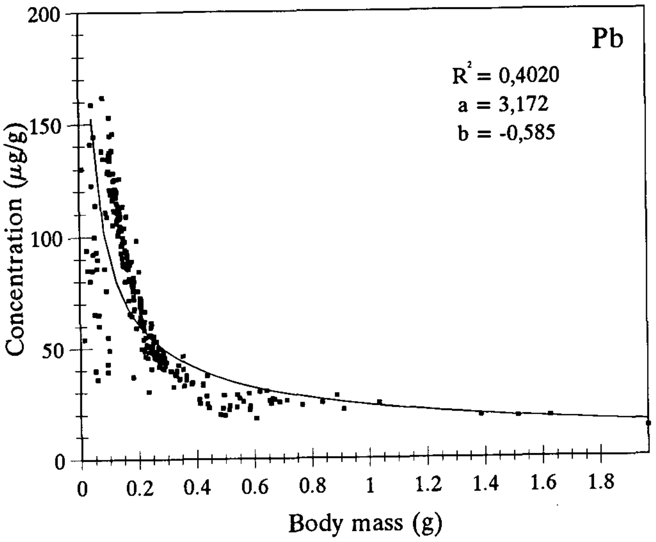

Figure 7
Relationship between lead concentration (µg/g) and body mass of P. philander *expressed in dry mass (g)*

tions of the metals in the fish followed almost the same pattern as those obtained for the sediments, namely Fe occurring in the highest concentrations (595 μg/g), followed by Zn (279 μg/g), Mn (99 μg/g), Ni (80 μg/g), Pb (μg/g) and Cu (9 μg/g). In the fish/sediment BCRs, an exceptionally high concentration factor was obtained for zinc (8.54). By contrast, a very low BCR of 0.1 was recorded for iron, while the BCRs for copper, manganese, nickel and lead were all within the same order of magnitude, fluctuating between 1.23 and 1.96 (Table 2).

Overall, a relatively wide range in concentrations was obtained for all metals in the individual fish samples (Table 2 and Figs. 2 to 7), with the smaller fish clearly having higher concentrations of metals per equivalent mass compared to those of the larger specimens. The greatest differences between the highest and lowest concentrations obtained for specific metals per fish mass were those for nickel, ranging between 7 μg/g and 333 μg/g, and iron, ranging between 105 μg/g and 3246 μg/g, lead (10 μg/g and 162 μg/g), manganese (41 μg/g and 549 μg/g), and copper (4 μg/g and 26 μg/g). The smallest difference between the highest and lowest concentration obtained for the fish for any metal was that for zinc, which varied between 154 μg/g and 549 μg/g.

An overall negative correlation for all metals examined was found to exist between the increase in mass of the individual fish and the metal concentration per equivalent mass (Table 3). The highest negative correlation was obtained for lead, followed by copper, zinc and nickel. The inverse relationships between metal concentration and the individual mass of the fish for the total population of *P. philander* were highly significant for all the metals (Table 3). According to Figs. 2 to 7, the plotted metal concentration:body mass ratios followed the contours of a power regression with the highest negative correlation occurring amongst the smaller fish between the mass groups of 0.008 g and 0.2 g. This tendency clearly declines with the increase in the mass and consequently age of the fish beyond 0.4 g (Figs. 2 to 7).

Discussion

Based on observations on its abundance in the impoundment *P. philander* does not appear to be detrimentally affected by the metal loads in the lake. This species is a benthic feeder and probably accumulates most of its metals via food, but also from the water environment where the metals in their ionic form can be absorbed through the gills and to a lesser extent through the skin surfaces of the fish (Villegas-Navarro and Villarreal-Trevino, 1989; Heath, 1987). Benthic macro-invertebrate fauna, the major food source of *P. philander*, is known for its ability to accumulate large quantities of metals from the sediments (Cover and Wilhm, 1982).

According to Heath (1987), a limited control mechanism for the uptake of essential metals occurs amongst fish, and elimination rates may be more dependent upon uptake rates (Bryan, 1964; 1967) than is probably the case for non-essential metals such as lead. With the exception of Pb, the other metals examined here are all essential for various respiratory and metaloenzymatic processes (Mohamed et al., 1990). The comparatively low BCR obtained for Fe could partially be explained on the basis of this control mechanism. BCRs obtained for all the other metals, which were present in much lower concentrations than Fe in both the water and sediments (Table 2), were relatively higher, fluctuating between 1.23 (Cu) and 8.54 (Zn). The BCR obtained for Pb, which was in the same order of magnitude as some of the essential metals examined, can possibly be explained by the fact that the main deposition site of this metal is bone, where it can substitute for Ca (Settle and Patterson, 1980; Miyahara et al., 1983; Sorensen, 1991). Lead uptake by the fish might, however, be restricted by the fact that this metal is largely absorbed through the gills and skin (Varanasi and Gmur, 1978; Moore and Ramamoorthy, 1984), and in particular with increasing dietary or aqueous Ca where Ca suppresses the synthesis of Ca-binding protein in the gut, resulting in reduced uptake of both Ca and Pb (Varanasi and Gmur, 1978).

Lead is known to be a cumulative toxic metal with no beneficial or nutritional qualities for fish or other vertebrates (McKee and Wolf, 1963). Lead in high enough concentrations can cause the inhibition of ALA-D activities in fish which results in anaemia (Hernberg et al., 1972; Johansson-Sjobeck and Larsson, 1979; Tewari et al., 1987). This metal also impairs the functions of the liver, kidney and spleen (Haider, 1964), and may cause a decrease in bone Ca (Dwyer et al., 1988) which may result in spinal deformities (Davies and Everhart, 1973) and even the death of fish (McKee and Wolf, 1963).

Looking at the juvenile fish's ability to accumulate higher concentrations of the metals compared to larger individuals, *P. philander* clearly displays a higher negative correlation between body mass and metal concentrations per mass unit amongst the smaller fish. These findings confirm those of O'Rear (1971) and Matthiessen and Brafield (1977) for the fish species *Morone saxatilis* (Cu and Zn) and *Gasterosteus aculeatus* (Zn). The distinctly negative correlation between body size and metal uptake amongst the smaller fish, especially those below 0.2 g in mass (Figs. 2 to 7), can most likely be related to the relatively higher oxygen consumption and metabolic processes amongst the smaller juvenile fish (Matthiessen and Brafield, 1977; Anderson and Spear, 1980; Gobas and Mackay, 1987; Clark et al., 1990).

The highest negative correlation between body mass and metal concentrations was obtained for Pb. Lead uptake under natural conditions might be directly linked to the oxygen consumption in fish as was shown for Zn-65 by Matthiesen and Brafield (1977). Furthermore, smaller fish have a disproportionately large mass-specific lamellar surface to volume ratio (Muir, 1969; Hughes, 1970), so that the juvenile *P. philander* may be able to bioconcentrate relatively more of the metal. In the case of the other metals examined, where uptake is to a large extent also dependent on feeding behaviour, more variation can be expected to occur because of feeding preferences and the health status of the individual fish.

Regulation mainly exists through the ability of fish to excrete the metals (Heath, 1987). It is possible that the inverse relationship between mass and metal concentrations that was found to exist for *P. philander* is a reflection of this fish's ability to bioregulate metals; i.e. larger individuals are better able to bioregulate or excrete the metal than smaller individuals. Somewhat contradictory findings exist in the literature in this respect. Anderson and Spear (1980) showed that a disproportionate decrease in Cu clearance rate occurs with increasing body mass in fish, so that smaller fish are potentially better able to excrete the metal than larger individuals. This was also found by Sharpe et al. (1977) for methylmercury clearance in goldfish, *Carassius auratus*. Matthiessen and Brafield (1977), however, showed in their uptake rate experiments of Zn by *Gasterosteus aculeatus* that the bioregulation mechanisms are triggered sooner in the larger specimens than in the smaller individuals, which might provide another possible explanation for the higher build-up in concentrations of the various metals concerned amongst the smaller fish of *P. philander*.

Conclusions

The present study showed that *P. philander* appears to be reasonably tolerant to various concentrations of the metals Fe, Mn, Zn, Cu, Ni and Pb in the Spaarwater Pan and that a possible mechanism exists whereby concentrations of these metals can be bioregulated, particularly so in the larger older fish of the population. This may explain its relative abundance in the Spaarwater Pan despite the constant discharge of mine seepage water into this water body.

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