Bioaccumulation of iron in the freshwater crab (*Potamonautes warreni*) from three industrial, mine and sewage polluted freshwater ecosystems in the Transvaal

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Abstract

An investigation was made into the bioaccumulation of iron in the organs and tissues of the freshwater crab *Potamonautes warreni* (Calman) from 3 metal-polluted aquatic ecosystems. Differences in water and sediment iron concentrations were related to environmentally induced factors. The highest iron concentrations in the crab occurred in the gills, suggesting this organ to be the prime site for the absorption and/or loss of iron to/from the aquatic environment. Although no seasonal or gender-related tendency in iron concentrations in the various organs and tissues was detected, there appears to be an inverse relationship in the capacity of the crab to bioaccumulate iron with size. A brief discussion is given on the physical and chemical conditions which prevailed at the 3 sampling sites.

Introduction

Iron is a metal which is abundant in nature, constituting 5% of the earth's crust (Friberg et al., 1986). The concentration of this metal can, however, drastically increase in surface waters due to various anthropogenic activities (Moore and Ramamoorthy, 1984). Although iron is vital for most living organisms, it is potentially harmful in all dosages and forms (Friberg et al., 1986). The importance of iron lies in its remarkable capacity to engage in electron transport reactions in biological systems (Neilands, 1974). Ferric hydroxide flocs have been observed to coat the gills of white perch, *Morone americanus*, minnows and silversides, *Menidia* sp. The smothering effects of settled iron precipitates may be particularly detrimental to fish eggs and bottom-dwelling fish food organisms (Train, 1979). Increased exposure of the banded tilapia (*Tilapia sparrmanii*) to iron has been shown to cause an increase in the total white blood cell counts suggesting a stimulation of the immune system to protect the organism against infections which may occur due to iron-mediated damage of the gill tissue (Wepener et al., 1992). However, information on the bioaccumulation of iron in the tissues and organs of decapods is limited in spite of the essential function of this metal in biological systems.

The present study was aimed at determining the iron concentrations in the water, sediments and in selected tissues and organs of the freshwater crab *P. warreni* from three different aquatic environments in the Transvaal, which have been subjected to various degrees of metal contamination emanating from different sources of mine, industrial and sewage pollution. Attempts were made to evaluate seasonality, gender and size of the crab in relation to the bioaccumulation of iron in selected organs and tissues of this organism. *P. warreni* was also considered as a possible indicator organism for iron contamination of freshwater ecosystems.

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Study area

The catchment of the Elsburgspruit-Natalspruit River system covers about 225 km^2. The headwater regions of these rivers are characterised by mine dumps, mine tailing dams, industries and sewage purification works. Because of these intensive mining, domestic and industrial activities this system is severely polluted in some places. Where the Elsburgspruit tributary joins the Natalspruit River (28°10'S:26°19'E), a large expanded wetland ecosystem of almost 8 km is formed (Fig. 1). Six localities with varying metal input were chosen in this wetland ecosystem for the sampling of *P. warreni*.

Crabs were also sampled from the Bronkhorstspruit River (28°41'S:26°00'E) near Delmas, Transvaal, as well as from the Nooitgedacht Dam (28°01'S:25°31'E) in Bophuthatswana (Fig. 1). The Bronkhorstspruit River flows mainly through agricultural areas, but is also subjected to urban, industrial and mining effluents, but to a much smaller extent than the Natalspruit River. The Nooitgedacht Dam is a shallow 10 ha man-made lake with a mean depth of 2,5 m (Fig. 1). The lake is subjected to limited domestic, agricultural and industrial effluents.

Materials and methods

Water and sediment samples for heavy metal and physico-chemical analyses were collected every second month (usually between 9:00 and 12:00) from the 3 sampling sites mentioned. Water samples were collected in well-rinsed 1 ℓ acid-washed polyethylene bottles approximately 10 cm below the surface. Sediment samples were taken from the river bottom (top 5 cm) and at the Nooitgedacht Dam, near the edge of the dam in the shallow water. In the laboratory the water and sediment samples were kept at approximately -4°C until analysed.

During each survey the temperature (°C), conductivity (µS/cm), pH and dissolved oxygen (mg/ℓ) were determined directly at the sampling site. The first 3 variables were measured with the aid of Hannah-instruments, while the oxygen was determined with an OXI-96 WTW oxygen meter. Physico-chemical analyses were done with the aid of a Hach model DR-EL/4 engineering kit,

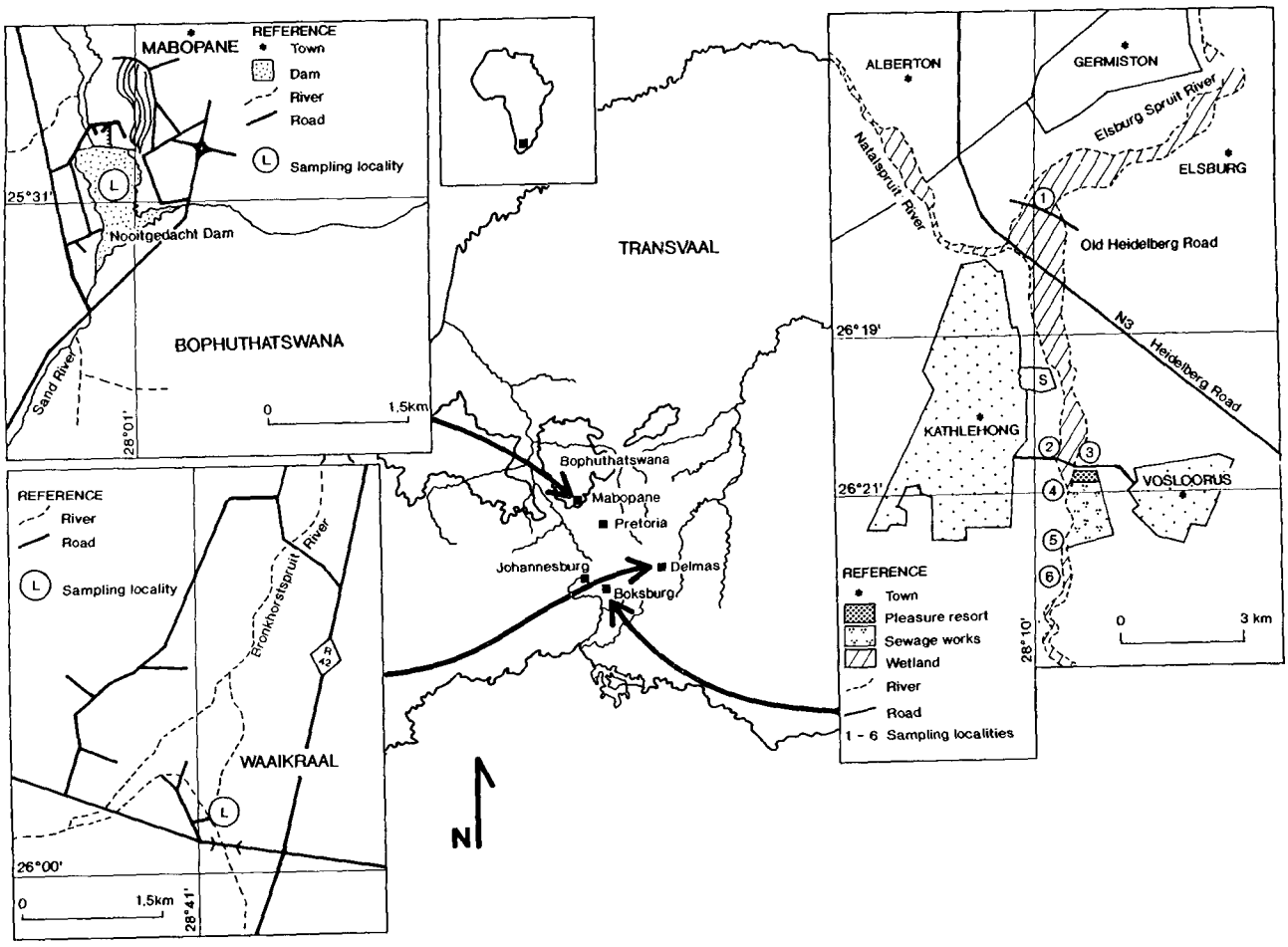

Figure 1
Sampling localities on the Natalspruit River wetland, the Bronkhorstspruit River and the Nooitgedacht Dam, South Africa

according to procedures described by *Standard Methods* (1989). The water samples were also used for heavy metal analyses.

The total metal concentration (dissolved, colloidal or particulate) in the water sample was determined by adding 5mℓ concentrated nitric acid, 5 mℓ concentrated perchloric acid and 40 mℓ well-mixed sample water into a 100 mℓ Erlenmeyer flask. It was then evaporated on a hotplate to approximately 5 ml. After digestion, each sample was made up to 40 mℓ with double distilled water (Van Loon, 1980).

Sediment samples were thawed and then dried at 100°C for 24 h. One gram (accurate to 0,01mg) of a finely ground, homogenised sediment sample was weighed into a 100 mℓ Erlenmeyer flask to which 20 mℓ concentrated nitric acid and 10 mℓ perchloric acid were added. The acid digestion was performed on a hotplate (200 to 250°C) for at least 5 h, during which clearing of the sample was achieved (Van Loon, 1980). Each sample was then made up to 50 mℓ with double distilled water. All the samples were stored in pre-washed bottles for later iron analyses.

Specimens of *P. warreni* were collected by hand and with baited traps at the mentioned localities every second month over a period of 14 months, from November 1989 to January 1991. The crabs were transported in polyethylene buckets to the laboratory, where samples of the carapace, gills, gonads, midgut gland and muscle were removed. In order to prevent contamination, the dissections of the organs and tissues were carried out using stainless steel tools on a stainless steel work surface. Prior to dissection, carapace width (mm) and gender were recorded for each crab. The crabs were grouped into 4 size-classes, according to carapace width: 10 to 30 mm (Group 1), 30 to 40 mm (Group 2), 40 to 60 mm (Group 3) and 60 to 80 mm (Group 4). Of these, Groups 1 and 2 both represented the immature developmental stages of *P. warreni*. After dissection 1 g of each tissue was stored in pre-washed glass tubes and kept at -4°C for later analysis.

Thawed samples were digested according to methods described by Van Loon (1980) and Du Preez and Steyn (1992), using 55% nitric acid and 70% perchloric acid in a ratio of 2:1. Due to small sample mass, it was necessary to pool some of the tissues of specimens of the 2 smaller size groups (Groups 1 and 2) to obtain the required 1 g wet tissue sample.

The iron concentration (accurate to 0,003 mg/ℓ) in the various organs and tissues, water and sediment was measured by means of flame atomic absorption spectrophotometry (Varian SpectrAA-10) (Friberg et al., 1986). Analytical standards were prepared from stock solutions of Holpro chemicals. The metal concentrations in the tissues were recalculated and expressed as μg metal/g wet tissue mass. In order to be able to facilitate comparison of dry mass data in the literature with the data obtained in this study, the dry matter percentages were determined in each case. The organs and tissues of 8 crabs were dissected and dried at 60°C for 48 h.

A bioaccumulation factor (BF) which reflects the difference in

TABLE 1
MEAN VALUES FOR PHYSICAL-CHEMICAL VARIABLES, AS WELL AS IRON CONCENTRATIONS IN THE WATER AND SEDIMENTS, DETERMINED OVER THE 14-MONTH SAMPLING PERIOD (NOVEMBER 1989 TO JANUARY 1991), AT THE NATALSPRUIT RIVER (LOCALITIES 1-6), BRONKHORSTSPRUIT RIVER AND NOOITGEDACHT DAM

		Natalspruit River 1	2	3	4	5	6	Bronkhorst-spruit River	Nooitgedacht Dam
Temperature (°C)	x̄±SD	16,5±5,0	19,1±6,0	18,4±6,0	18,1±5,7	25,1±0,9	20,8±5,7	18,8±4,8	22,9±5,3
	Range	9,0-21,0	10,0-28,0	9,0-26,5	9,0-24,3	24,2-26,0	12,0-26,6	11,0-23,5	15,0-28,0
pH	x̄±SD	7,1±0,4*	7,7±0,3*	7,4±0,4*	7,4±0,5*	8,2±0,1*	7,6±0,4*	8,1±0,3*	8,2±0,3*
	Range	6,8-7,9	7,4-8,2	6,8-8,0	6,7-8,3	8,2-8,3	7,1-8,5	7,8-8,5	8,0-8,8
Conductivity (µs/cm)	x̄±SD	1757,5±409,1	1806,3±399,7	1325,0±216,9	1395,0±189,7	700,0±445,4	1262,5±165,2	301,4±51,5	331,4±31,3
	Range	830,0-2000,0	1200,0-2300,0	1030,0-1690,0	1090,0-1600,0	220,0-1100,0	1030,0-1470,0	250,0-390,0	290,0-370,0
Dissolved oxygen (mg/ℓ)	x̄±SD	9,8±2,8	8,4±3,0	8,0±1,9	8,9±1,1	6,2±0,0	10,1±3,5	9,9±4,6	9,3±2,5
	Range	6,8-12,9	6,1-13,4	6,0-10,6	7,7-10,7	-	5,8-13,6	3,7-13,8	6,5-13,3
Total hardness (mg/ℓ $CaCO_3$)	x̄±SD	519,6±262,4	525,3±302,6	440,1±129,1	460,8±162,4	150,0±55,5	347,4±42,8	111,3±37,1	82,1±29,3
	Range	270,0-1003,0	260,0-1090,0	328,0-711,0	220,0-690,0	116,0-214,0	300,0-412,0	80,0-189,0	42,0-132,0
Alkalinity (mg/ℓ $CaCO_3$)	x̄±SD	74,5±26,4	163,4±61,6	68,3±23,4	74,8±37,2	39,3±19,7	69,8±33,3	86,0±43,6	61,1±22,1
	Range	40,0-122,0	54,0-224,0	42,0-114,0	38,0-128,0	26,0-62,0	38,0-142,0	58,0-176,0	32,0-96,0
Ammonium (mg/ℓ NH3)	x̄±SD	0,89±0,34	1,25±0,73	1,91±0,91	1,71±0,86	1,29±1,20	1,58±0,55	0,60±0,01	0,81±0,63
	Range	0,52-1,34	0,48-2,56	0,73-3,42	0,84-3,05	0,59-2,68	0,93-2,26	0,51-0,72	0,39-2,20
Nitrate (mg/ℓ NO_3)	x̄±SD	6,10±2,30	6,30±2,30	6,03±3,12	5,13±2,73	8,85±5,32	8,74±2,92	5,85±2,61	3,28±1,56
	Range	3,52-10,12	3,96-10,56	3,21-13,20	2,94-11,0	5,28-14,96	5,90-12,76	2,64-11,2	1,90-6,00
Nitrite (mg/ℓ NO_2)	x̄±SD	0,06±0,07	0,03±0,01	0,31±0,23	0,29±0,28	0,43±0,27	0,57±0,26	0,07±0,1	0,15±0,13
	Range	0,01-0,20	0,01-0,05	0,05-0,66	0,05-0,89	0,21-0,73	0,17-0,92	0,01-0,30	0,03-0,38
Phosphate (mg/ℓ PO_4)	x̄±SD	1,04±0,69	0,70±0,30	1,50±0,20	1,04±0,34	1,32±1,11	1,90±0,70	0,27±0,21	0,27±0,63
	Range	0,21-1,63	0,32-1,25	1,10-1,70	0,50-1,40	1,64-2,60	0,34-2,80	0,02-0,69	0,02-1,70
Sulphate (mg/ℓ SO_4)	x̄±SD	169,4±5,6	169,3±50,2	167,5±10,0	169,4±7,3	123,3±35,5	160,0±16,9	2,4±2,6	6,3±7,9
	Range	160,0-180,0	84,0-270,0	160,0-190,0	160,0-180,0	85,0-155,0	120,0-175,0	0,05-7,0	0,05-22,0
[Fe] in water (mg/ℓ)	x̄±SD	1,81±0,23	1,75±0,33	3,38±1,06	1,85±0,35	3,00±0,18	1,82±0,18	3,25±0,96	2,06±0,15
	Range	1,56-2,01	1,37-2,28	1,96-4,78	1,24-2,45	2,90-3,26	1,56-2,02	2,01-4,43	1,84-2,25
[Fe] in sediment (µg/g)	x̄±SD	76557,1±49727,1	23935,7±10718,7	38050,0±18068,3	48764,3±27173,7	49400,0±1697,1	39242,9±34158,6	21650,0±21702,5	37921,4±29842,4
	Range	8600,0-164000,0	14600,0-47350,0	17200,0-43950,0	13100,0-98450,0	48200,0-50600,0	8250,0-100150,0	6650,0-65950,0	12550,0-42600,0

* Mean of - log [H+]

TABLE 2
THE MEAN CONCENTRATION OF IRON (µg/g WET MASS) IN *POTAMONAUTES WARRENI* SAMPLED FROM THE NATALSPRUIT RIVER (LOCALITIES 1-6), THE BRONKHORSTSPRUIT RIVER (BS) AND THE NOOITGEDACHT DAM (ND). THE BIOACCUMULATION FACTOR (BF) WITH RESPECT TO THE WATER AND SEDIMENT IS ALSO GIVEN. DRY MATTER PERCENTAGES: CARAPACE= 80,9% DRY MASS; GILLS=13,2% DRY MASS; GONADS=39,3% DRY MASS; MIDGUT GLAND=32,6% DRY MASS AND MUSCLE=19,6% DRY MASS

Localities		Carapace	Gills	Gonads	Midgut gland	Muscle
1	n	63	57	49	56	62
	$\bar{x}$ ±SD	121,8±59,0	182,7±106,2	117,8±96,6	258,8±573,0	82,8±42,1
	Range	13,5-321,5	54,0-563,0	35,0-713,6	18,8-2815,0	29,5-268,5
	BF (H_2O)	67,3	100,9	65,1	143,0	45,7
	BF (Sediment)	<0,1	<0,1	<0,1	<0,1	<0,1
2	n	128	129	68	120	121
	$\bar{x}$ ±SD	135,0±119,0	223,8±178,9	228,2±311,1	170,3±157,0	94,7±110,6
	Range	38,3-975,0	46,5-1585,0	27,0-1340,0	31,5-1277,0	21,0-820,0
	BF (H_2O)	77,1	127,9	130,4	97,3	54,1
	BF (Sediment)	<0,1	<0,1	<0,1	<0,1	<0,1
3	n	65	65	47	61	63
	$\bar{x}$ ±SD	176,7±132,1	337,5±180,0	111,3±49,9	207,8±171,9	98,2±54,7
	Range	51,0-705,0	50,5-1075,0	33,3-245,0	69,5-1100,0	20,5-270,0
	BF (H_2O)	52,3	99,9	32,9	61,5	29,1
	BF (Sediment)	<0,1	<0,1	<0,1	<0,1	<0,1
4	n	110	110	33	103	105
	$\bar{x}$ ±SD	140,9±72,8	223,2±170,0	138,1±172,9	297,8±433,2	103,8±92,4
	Range	40,0-465,0	35,0-913,4	37,5-980,0	38,5-2835,7	20,0-755,0
	BF (H_2O)	76,2	120,6	74,6	160,9	56,1
	BF (Sediment)	<0,1	<0,1	<0,1	<0,1	<0,1
5	n	24	26	6	17	22
	$\bar{x}$ ±SD	186,7±84,3	229,3±223,1	135,1±61,6	143,2±104,6	117,6±131,6
	Range	85,5-370,0	76,0-950,0	75,8-215,0	6,1-461,5	36,0-652,2
	BF (H_2O)	60,2	73,9	43,6	46,2	37,9
	BF (Sediment)	<0,1	<0,1	<0,1	<0,1	<0,1
6	n	84	84	38	83	80
	$\bar{x}$ ±SD	138,1±81,6	286,9±310,6	223,9±240,6	246,2±239,5	114,6±160,8
	Range	41,0-382,1	38,5-2130,0	29,0-1130,0	42,5-1405,0	28,0-1123,1
	BF (H_2O)	75,9	157,6	123,0	135,3	62,9
	BF (Sediment)	<0,1	<0,1	<0,1	<0,1	<0,1
BS	n	113	110	43	91	104
	$\bar{x}$ ±SD	209,8±97,6	392,3±371,0	249,5±202,9	498,4±526,1	171,7±212,2
	Range	90,0-570,5	80,5-3650,0	68,0-850,0	81,5-3315,0	34,0-1436,4
	BF (H_2O)	64,6	120,7	76,8	153,4	52,8
	BF (Sediment)	<0,1	<0,1	<0,1	<0,1	<0,1
ND	n	144	143	79	142	140
	$\bar{x}$ ±SD	144,6±70,2	371,6±245,5	135,1±120,1	261,8±248,9	75,9±51,8
	Range	56,0-576,0	4,6-1500,0	34,0-825,0	50,0-1540,0	27,5-480,0
	BF (H_2O)	70,2	180,4	65,6	127,1	36,8
	BF (Sediment)	<0,1	<0,1	<0,1	<0,1	<0,1

concentration of the metal in the water and the tissue was calculated using the formula of Wiener and Giesy (1979):

$$BF = Co/Cw$$

where:

Co = wet mass concentration of a given metal in the crab tissue
Cw = the average total concentration of the metal in the water.

A bioaccumulation factor with respect to the sediment was also determined. The formula of Kovacs et al. (1984) was used:

BF = [metal] in the tissue/[metal] in the sediment.

Statistical analysis was performed on the data with the aid of the BMDP2V program. If statistically significant differences were found after analysis of variance (ANOVA), Sheffe's test for paired comparison was performed on the data. Student's t-test was used to indicate the extent of significant differences which may possibly exist between male and female *P. warreni* in the bioaccumulation of iron.

Results

Water and sediment

Over the 14-month sampling period between November 1989 to January 1991, temperatures ranged between 16,5 to 25,1°C in the Natalspruit River. Temperatures at Localities 5 and 6 were higher in comparison with the other four localities (Table 1). Temperatures measured in the Nooitgedacht Dam were much higher (22,9±5,3°C) than those recorded in either the Natalspruit (19,0±5,6°C) or Bronkhorstspruit Rivers (18,8±4,8°C).

The pH of the Natalspruit River water was alkaline, ranging from 7,1 to 8,2 with a calculated mean of 7,5. The mean pH values measured for the Bronkhorstspruit River and Nooitgedacht Dam were 8,1 and 8,2 respectively, indicating more alkaline conditions in the latter 2 systems. The conductivity of the water in the Natalspruit River system was high, with an average of 1452,5±418,3 µS/cm, compared to those of the Bronkhorstspruit River (301,4±51,5 µS/cm) and Nooitgedacht Dam (331,4±31,3 µS/cm), respectively (Table 1).

In contrast to the Natalspruit River the total hardness measured at the Bronkhorstspruit River and Nooitgedacht Dam was relatively low (Table 1). The mean alkalinity of the waters of the different localities at the Natalspruit River varied between 39,3±49,7 mg/*l* and 163,4±61,6 mg/*l*. The mean value for the Bronkhorstspruit River and the Nooitgedacht Dam was 86,0±43,6 mg/*l* and 61,1 ±22,1 mg/*l*, respectively (Table 1).

Values obtained for dissolved oxygen indicate that all 3 aquatic systems were well oxygenated, throughout the study period. The average oxygen levels measured in the Natalspruit River were 8,9 mg/*l*, 9,9 mg/*l* in the Bronkhorstspruit River and 9,3 mg/*l* in the Nooitgedacht Dam. Results obtained for ammonium, nitrate, nitrite and phosphate indicated that the Bronkhorstspruit River and the Nooitgedacht Dam were comparatively low in concentrations of nitrogen and phosphate. The ammonia and nitrate values obtained for the Natalspruit River, however, were relatively high, fluctuating between 0,89 and 1,91 mg/*l* and 5,13 and 8,85 mg/*l*, respectively. The sulphate values obtained for the Natalspruit River were particularly high (163,8±26,7 mg/*l*) compared to concentrations in the Bronkhorstspruit River (2,4±2,6 mg/*l*) and the Nooitgedacht Dam (6,3±7,9 mg/*l*) (Table 1).

The highest iron concentrations in the water were recorded in the Bronkhorstspruit River (3,25±0,96 mg/*l*), while the iron concentration in the Natalspruit River was 2,19±0,83 mg/*l* and 2,06±0,5 mg/*l* in the Nooitgedacht Dam. Fluctuations in the concentrations of iron between the different months for the same localities were relatively low. The iron concentrations in the sediments of the Bronkhorstspruit River, however, were the lowest (21 650,0± 21 752,5 µg/g) in comparison with the iron concentrations in the Natalspruit River (45 531,1±33 205,5 µg/g) and the Nooitgedacht Dam (37 921,4±29 842,4 µg/g) (Table 1).

Bioaccumulation of iron in the organs and tissues of the crab

The values for iron in the different organs of crabs sampled from all the localities were pooled to determine tissue variation in the bioaccumulation of iron within and between the tissues. With the exception of the concentration between the gills (303,0±227,2 µg/g wet mass) and the midgut gland (mean: 245,8±363,8 µg/g wet mass), there were significant differences ($P<0,05$) in the iron concentration among all the other organs and tissues of *P. warreni*. The lowest mean iron concentration was found in the muscle tissues (87,8±87,2 µg/g wet mass), while the carapace and gonad iron concentration of 137,2±88,5 µg/g wet mass and 172,0±200,3 µg/g wet mass, respectively, were amongst the highest.

There were no significant differences ($P>0,05$) in the iron concentration in the various tissues and organs of crabs sampled from the different localities in the Natalspruit River (Table 2). The carapace iron concentration in crabs sampled from the Bronkhorstspruit River was, however, significantly higher ($P<0,05$) than in crabs sampled from Localities 1, 2, 4, 6 (Natalspruit River) and the Nooitgedacht Dam. The gill iron concentration in crabs sampled from the Nooitgedacht Dam and the Bronkhorstspruit River was significantly higher ($P<0,05$) than in crabs from Localities 1, 2 and 4 (Natalspruit River), while the midgut gland iron concentration in *P. warreni* from the Bronkhorstspruit River was significantly higher ($P<0,05$) than from the other 2 localities. Accordingly, the muscle iron concentration of crabs from the Bronkhorstspruit River was significantly higher ($P<0,05$) than those of crabs sampled from localities 1, 2, 3, 4 (Natalspruit River) and from the Nooitgedacht Dam.

Crabs from the Bronkhorstspruit River had a significantly higher ($P<0,05$) carapace, gonad, midgut gland and muscle iron concentration than crabs from both the Natalspruit River (pooled localities) and the Nooitgedacht Dam (Fig. 2). The gill iron concentration in crabs from the Natalspruit River was significantly lower ($P<0,05$) than concentrations in crabs from either the Bronkhorstspruit River or the Nooitgedacht Dam.

The bioaccumulation factor (BF) with respect to iron contamination in the water was highest in the gills and the midgut gland, ranging from 73,3 to 180,4 and 46,2 to 160,9 respectively, while the lowest BF values were recorded for the muscle (29,1 to 62,9). The BF with respect to iron in the sediment was consistently less than 0,1 for all the tissues and organs (Table 2).

No specific seasonal or gender-related trends were observed in the bioaccumulation of iron in the different tissues and organs of *P. warreni*. In crabs sampled from the Natalspruit River, however, there were significant differences ($P<0,05$) among the different size groups in the bioaccumulation of iron in the carapace, gonads, midgut gland and muscle, respectively. The iron concentration in the carapace of crabs from Group 1 was significantly higher ($P<0,05$) than the concentration in the carapace of crabs from Groups 3 and 4. The gonad iron concentration in *P. warreni* from Group 3 was significantly higher ($P<0,05$) than that in crabs from

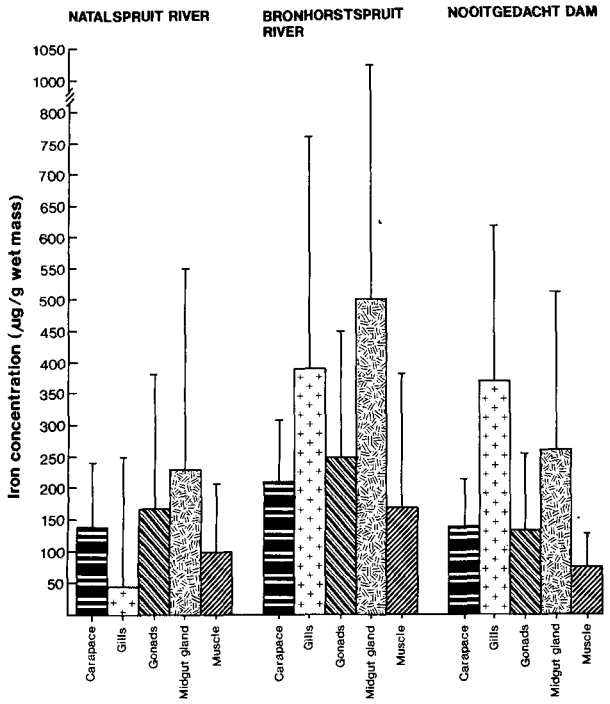

Figure 2
The mean concentration ($\bar{x} \pm SD$) of iron (µg/g wet mass) in the selected tissues and organs of Potamonautes warreni *sampled from the Natalspruit River (pooled localities), Bronkhorstspruit River and Nooitgedacht Dam*

Group 4. The midgut gland iron concentration in crabs from Group 1 was significantly higher (P<0,05) than that in crabs from Group 4.

There was a consistent increase in iron concentration in the muscle with a decrease in size (Table 3). The iron concentration in the muscle of crabs from Group 1 was significantly higher (P<0,05) than in the other size groups, while the iron concentration in the crabs from Group 2 was significantly higher than in crabs from Group 4. A general rise in the tissue iron concentration occurred with a decrease in carapace width in crabs sampled from the Natalspruit River (Table 3).

Crabs from the Bronkhorstspruit River showed significant differences (P<0,05) in the gill, gonad, midgut gland and muscle iron concentration among the different size groups. The iron concentration in the gills and muscle of crabs from Group 1 was significantly higher (P<0,05) than that in the tissues of the rest of the size groups, while the gonad and midgut gland iron concentrations in crabs from Group 3 were significantly higher (P<0,05) than that in crabs from Group 4 (Table 3). The only significant difference (P<0,05) detected in the bioaccumulation of iron between the different size groups of crabs sampled from the Nooitgedacht Dam was in the carapace, where the concentration in crabs from Group 1 was higher than the concentration in crabs from Group 4 (Table 3). In the crabs sampled from the latter 2 systems, there was also a tendency for immature *P. warreni* to bioaccumulate iron to higher levels than in mature crabs, although this trend was not as apparent as in crabs sampled from the Natalspruit River.

Discussion

Higher water temperatures measured at Localities 5 and 6 of the Natalspruit River can be related to the fact that water from the last maturation pond of the Boksburg Sewage Purification Works reaches the river at Locality 5. Differences in temperatures measured at the Nooitgedacht Dam and the Bronkhorstspruit River can be attributed to the geographical location of these aquatic systems. The Nooitgedacht Dam is situated in a relatively warm area, while generally lower temperatures prevail in the Bronkhorstspruit River area. This is important in that concentration rates and uptake of heavy metals increase with increasing temperatures (Cairns et al., 1975).

In the present study the pH of the water at the Bronkhorstspruit River and the Nooitgedacht Dam never declined below 7,8, while the pH values obtained in the Natalspruit River varied considerably (6,7 to 8,5). This variation can partially be attributed to acid rock drainage, to algal activity, as well as to mining and industrial activities in the catchment area. The conductivity of the water at Localities 1 and 2 (Natalspruit River) was consistently high. These 2 localities receive high loads of metal and mineral ions from mining and industrial areas in the upstream region of this system. From here, the water flows through an expanded wetland ecosystem, purifying it to a certain extent. The high conductivity values measured at Locality 6 reflect the combined effects of effluents from the sewage purification works and from stormwater drains from surrounding townships. As a result of the various anthropogenic activities in the Natalspruit River catchment area the conductivity of the water is generally higher in this system than those measured in either the Bronkhorstspruit River and the Nooitgedacht Dam. The toxicity of iron to aquatic life is, however, higher in water with a low conductivity and low pH (Kempster et al., 1980). The total hardness measured at the Natalspruit River was relatively high. The toxicity of metals may be influenced by the hardness of the water. This is related to the antagonistic effect of calcium and magnesium salts on the metals (Waldichuk, 1974). This effect is usually attributed to the complexing of the toxic cations and low toxicity of the resulting complexes (e.g. cupric carbonate) (Zitko and Carson, 1976). The toxic effect of metals in solution would therefore generally be reduced in the relatively hard water of the Natalspruit River, in contrast to the water of the Bronkhorstspruit River and the Nooitgedacht Dam which are both moderately hard-water systems.

The ammonia concentrations in the Bronkhorstspruit River and the Nooitgedacht Dam were generally lower than in the Natalspruit River, the latter being subject to a higher degree of urban runoff and sewage effluents than the other 2 systems. Under aerobic conditions, as found in all 3 systems, nitrifying bacteria predominate and ammonia is rapidly converted to the less toxic nitrite and, subsequently, to the relatively innocuous nitrate. Even though the Natalspruit River receives sewage effluents and one would expect high nitrogen values in this river, the values never exceeded the maximum stated criteria (124 mg/l) of Kempster et al. (1980).

Phosphate concentrations measured in the Natalspruit River were high compared to the values obtained from the Bronkhorstspruit River and the Nooitgedacht Dam, and although high phosphate concentrations are not toxic, they are important algal and plant nutrients and are to a certain extent indicative of pollution by detergents, fertilisers and sewage (Kempster et al., 1980). High sulphate values detected in the Natalspruit River are a direct result of the seepage and effluents from mines upstream in the catchment area of this river system. The degradation of organic matter in

TABLE 3

THE CONCENTRATION OF IRON (μg/g WET MASS) FOR THE FOUR SIZE GROUPS OF *POTAMONAUTES WARRENI*, WITH RESPECT TO THE DIFFERENT TISSUES AND ORGANS OF CRABS SAMPLED FROM THE NATALSPRUIT RIVER, BRONKHORSTSPRUIT RIVER AND NOOITGEDACHT DAM

Size groups		Carapace	Gills	Gonads	Midgut gland	Muscle
Natalspruit River						
1	n	40	38	-	34	33
	$\bar{x}$ ±SD	216,6±107,7	298,7±183,9		357,3±293,2	211,4±158,6
	Range	51,9-556,7	92,7-950,0		57,5-1208,3	23,5-775,0
2	n	49	52	-	47	43
	$\bar{x}$ ±SD	166,0±89,4	222,7±118,8		245,0±290,5	133,8±144,8
	Range	73,3-390,5	85,0-648,3		38,0-1500,0	21,0-820,0
3	n	135	132	43	126	129
	$\bar{x}$ ±SD	142,2±100,6	218,9±175,0	361±359,8	268,1±376,3	100,8±134,9
	Range	38,3-705,0	35,0-1075,0	29,0-1225,0	6,1-2835,7	21,0-1123,1
4	n	250	249	197	233	248
	$\bar{x}$ ±SD	128,1±91,7	257,0±237,7	125,5±132,2	187,5±284,2	79,4±46,0
	Range	13,5-975,0	44,0-2130,0	27,0-1340,0	18,8-2815,0	20,0-319,5
Bronkhorstspruit River						
1	n	12	11	-	10	11
	$\bar{x}$ ±SD	252,8±88,1	766,9±994,6		547,483,9	446,9±483,8
	Range	140,5-398,6	137,5-3650,0		94,0-1500,0	58,2-1436,4
2	n	15	14	-	16	11
	$\bar{x}$ ±SD	225,3±133,5	325,2±222,6		555,8±429,2	152,9±69,8
	Range	93,5-520,3	103,0-781,0		81,5-1495,0	51,0-296,3
3	n	36	36	15	26	32
	$\bar{x}$ ±SD	200,0±92,0	345,2±152,7	399,8±263,7	707,3±778,0	132,3±71,1
	Range	90,0-570,5	118,5-650,0	120,0-850,0	83,0-3315,0	40,0-398,7
4	n	50	49	28	39	50
	$\bar{x}$ ±SD	201,9±90,7	361,9±200,1	169,0±93,3	323,2±256,3	140,4±153,7
	Range	93,0-526,0	80,5-900,0	68,0-547,5	91,0-1475,0	34,0-905,0
Nooitgedacht Dam						
1	n	9	9	-	8	7
	$\bar{x}$ ±SD	207,9±63,6	294,1±111,1		280,5±132,8	78,7±29,7
	Range	139,0-338,0	142,0-435,0		150,0-561,0	61,0-145,0
2	n	12	12	-	12	12
	SD	166,3±45,0	346,3±190,7		301,3±180,3	76,4±40,1
	Range	80,5-244,5	96,0-637,1		170,2-800,0	34,5-140,5
3	n	47	47	11	46	47
	$\bar{x}$ ±SD	138,5±82,1	412,2±270,9	106,3±52,0	309,3±317,4	91,4±77,7
	Range	59,0-576,0	96,4-1500,0	57,1-235,0	70,0-1540,0	29,5-480,0
4	n	76	75	68	76	74
	$\bar{x}$ ±SD	137,5±62,4	359,4±247,4	139,7±127,4	224,8±215,8	65,8±27,1
	Range	56,0-364,0	4,6-1180,0	34,0-825,0	50,0-1500,0	27,5-138,5

– No data available

sediments may also contribute to high sulphate concentrations in the water (Hellawell, 1986).

Values for the 8 physical-chemical water quality parameters measured in these receiving aquatic ecosystems were all within the limits of the criteria for the protection of aquatic life as stated by Kempster et al. (1980). The total hardness, conductivity and sulphate concentrations were the only parameters which were abnormally high in the Natalspruit River, but this could be expected as the water of this wetland ecosystem consists of effluent waters from mines and industries in its headwater region. This corresponds with results obtained by Van Eeden (1990).

The high iron concentrations measured in the water and sediment of the Natalspruit River, Bronkhorstspruit River and Nooitgedacht Dam can be attributed to the mentioned anthropogenic activities in the respective catchment areas. It could also be partially due to natural iron-bearing formations. The iron concentration in the water of the Natalspruit River, Bronkhorstspruit River and Nooitgedacht Dam did not fall within the criteria for surface water for fish and aquatic life as stated by Kempster et al. (1980), Gardiner and Zabel (1989), Environment Canada (1987) and Hart (1974). This indicates that iron could potentially pose a threat to aquatic life in all 3 ecosystems. The concentration of iron in the water of the Natalspruit River during this study was consistently higher than concentrations obtained by Van Eeden (1990) for the same localities, while no changes could be observed in the corresponding metal concentration in the sediments.

It should be remembered, however, that the metal species, rather than the total concentration of a metal, is the key to its effects on the aquatic biota. The physico-chemical conditions in receiving waters may lead to a change in the speciation and toxicity of metals. Depending on environmental conditions, there could be a change in density, diversity, community structure and species composition of animal populations. The nature and extent of change depend largely on the concentration of heavy metal species in the water and sediment (Moore and Ramamoorthy, 1984).

Apart from physical and chemical water quality parameters there are a number of other factors which could influence the bioaccumulation of iron in the different tissues and organs of *P. warreni*, e.g. size differences, starvation, age, stage in life history, sex and moulting (Bryan, 1976; Skidmore, 1964). Variations in some physiological parameters due to genetic variation between the individual organisms may also play a role (Nugegoda and Rainbow, 1989). These factors may, however, have a much smaller influence on the concentrations of contaminants ultimately accumulated, since rates of loss may be equally affected (Bryan 1979).

Potamonautes warreni is able to bioaccumulate high levels of iron. Van Eeden and Schoonbee (1991) detected iron concentrations in whole *P. warreni* ranging between 158,7 to 1 068,8 µg/g dry mass. The data presented in this study showed that *P. warreni* was able to bioaccumulate iron to different levels in the different tissues and organs. Intraspecific variation in the bioaccumulation of iron was also observed. The iron concentration in the carapace was relatively low compared to the iron concentration in the other tissues. A significant correlation (r=0,89) was observed between the water iron concentration and the carapace iron concentration, indicating that the carapace may be useful as indicator tissue of iron concentration in the surrounding water column. It might also be possible that the carapace acts as a sink for excess iron absorbed from the surrounding medium. The highest mean iron concentration in *P. warreni* sampled from the 3 water bodies was detected in the gills. The latter appears to be the main site for the absorption and/or loss of metals across the body surface (Bryan, 1968). As with the carapace, a significant correlation (r=0,60) was found between the gill iron concentration and the water iron concentration.

In crustacea, digestive, storage and excretory organs seems to be the prime sites for the detoxification and storage of metals (Brown, 1982). The major metabolic organ of crustaceans is the midgut gland, which has been shown to function in food absorption and secretion of digestive enzymes, and which also serves as a storage depot for lipids, glycogen and minerals (Bruggren and McMahon, 1986). In addition, this organ is the principal site for the accumulation of most essential metal ions (Lyon et al., 1983). Alikhan et al. (1990) found iron concentrations in the midgut gland of *C. bartoni* ranging from 56 to 368 µg/g dry weight in water with an iron concentration varying between 46,7 and 98,71 µg/ℓ. Although much higher iron concentrations were found in the midgut gland of *P. warreni*, it should be remembered that the water iron concentrations from which these organisms were sampled were also much higher. Studies undertaken by Roldan and Shivers (1987) showed that the crayfish *Orconectes propinquus* naturally accumulate iron from their environment and store it in the midgut gland. It was suggested by these authors that metabolic processing of the ingested metal ions must occur during their passage through the cell interior involving a particular group of cytosolic proteins normally present in the midgut gland. These metal binding proteins complex with metal ions and mediate the transfer of the metal ions to lysosome-like vacuoles where they are compartmentalised (Lyon, 1984). The formation of intracellular vacuoles, recognised as secondary lysosomes (Brown, 1982), has been established as a mechanism for sequestration, detoxification and storage of metals in crustaceans (Coombs and George, 1978). The same mechanism may be imposed by *P. warreni* and may explain the high iron concentrations detected in the midgut gland. This mechanism prevents contact of excess metal with vital cell constituents and effectively detoxifies the metal until it is eliminated or passed on to other tissues.

The muscle of *P. warreni* appears to be the least preferred site for the bioaccumulation of metals as the lowest iron concentration was detected in this tissue. Despite this, correlations were observed between the muscle iron concentration and the water iron concentration (r=0,54), as well as between the muscle iron concentration and the sediment iron concentration (r=-0,86).

A tendency towards higher iron concentrations exists in *P. warreni* found in areas with a higher water iron concentration. The highest mean water iron concentration was measured in the Bronkhorstspruit River. Crabs sampled from this river had higher mean iron concentrations in the carapace, gonads, midgut gland and muscle than crabs sampled from either the Natalspruit River and the Nooitgedacht Dam which had lower water iron concentrations. Alikhan et al. (1990) suggested that the higher iron concentrations in the crayfish appear to be a function of the input of these metals into the habitat. Similar positive relationships between mean tissue concentrations of iron in aquatic crustacean species and their levels in the habitat were suggested by Anderson and Brower (1978).

The BF, calculated with respect to the water, was highest in the gills and midgut gland of *P. warreni*. This was to be expected since these 2 tissues appeared to be the prime sites for the detoxification and storage of metals (Brown, 1982). Although the iron concentration in the sediments of the Natalspruit River was very high compared to the other 2 sampling localities, this was not reflected in the tissue iron concentrations. The BF calculated for all the tissues, with respect to the iron concentration in the sediment, was consistently less than 0,1, indicating a comparatively low bioavailability of this precipitated iron. Because iron does not seem

to be well regulated in the freshwater crab and since there is a correlation between the iron concentration in the carapace, gills and muscle and the water iron concentration, it may be assumed that the BF provide some indication of the bioavailability of this metal in the aquatic ecosystem to *P. warreni*.

Although a significant rise in the bioaccumulation of iron was observed for some periods in crabs sampled from the different localities, no specific seasonal trend was observed. No significant rise was detected in the water and sediment iron concentrations during these periods, but it should be remembered that measurements were only taken once every 2 months, and that an accurate assessment of the iron concentrations in the environment could therefore not be obtained. There are also several other factors, such as rainfall, which may have influenced the concentration of this metal. No specific gender-related trends could be observed, not even in the gonads of mature organisms. The results obtained in the present study are, however, in accordance with results obtained by Anderson and Brower (1978) for the crayfish *Orconectes virilis*. These authors also found gender not to be a factor in the bioaccumulation of iron.

A general increase in the tissue iron concentration occurred with a decrease in the size of crabs. This trend was especially consistent in crabs sampled from the Natalspruit River. It appears that younger crabs are able to accumulate higher loads of iron than mature crabs, probably because of a higher metabolic rate, and the fact that detoxification processes are not yet well developed.

The observation that *P. warreni* accumulate high levels of iron in their various tissues and organs, and that they are not able to regulate the internal concentrations of iron as well as the concentration of other essential metals in the body, e.g. zinc (Du Preez et al., 1993), suggests that these freshwater crabs are potential indicators of iron pollution in aquatic systems. Significant correlations were detected between the water iron concentration and the carapace, gill and muscle iron concentrations. It is therefore proposed that these tissues of the crab be used as indicators of iron pollution in the aquatic habitat. However, more detailed experimental evidence is needed to support this hypothesis. It is suggested that specific experimental work on the variability of tissue iron concentrations be carried out under controlled laboratory conditions since there are many external factors (e.g. physical and chemical water quality parameters, moult-cycle of the test organism) which may influence the bioaccumulation of metals in the various tissues and organs of *P. warreni*.

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