Aquatic Toxicology 106–107 (2012) 76–84

Contents lists available at SciVerse ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aquatox

Parasites modify sub-cellular partitioning of metals in the gut of fish

Elijah Oyoo-Okoth[a,b,*], Wim Admiraal[b], Odipo Osano[a], Michiel H.S. Kraak[b], John Gichuki[c], Caleb Ogwai[c]

[a] Division of Environmental Health, School of Environmental Studies, Moi University, P.O. Box 3900, Eldoret, Kenya
[b] Department of Aquatic Ecology and Ecotoxicology, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, P.O. Box 9424/1090 GE, The Netherlands
[c] Kenya Marine and Fisheries Research Institute, P.O. Box 1881, Kisumu, Kenya

ARTICLE INFO

Article history:
Received 1 June 2011
Received in revised form 6 October 2011
Accepted 21 October 2011

Key words:
Fish
Ligula intestinalis
Metals
Parasites
Rastrineobola argentea
Subcellular distribution

ABSTRACT

Infestation of fish by parasites may influence metal accumulation patterns in the host. However, the subcellular mechanisms of these processes have rarely been studied. Therefore, this study determined how a cyprinid fish (*Rastrineobola argentea*) partitioned four metals (Cd, Cr, Zn and Cu) in the subcellular fractions of the gut in presence of an endoparasite (*Ligula intestinalis*). The fish were sampled along four sites in Lake Victoria, Kenya differing in metal contamination. Accumulation of Cd, Cr and Zn was higher in the whole body and in the gut of parasitized fish compared to non-parasitized fish, while Cu was depleted in parasitized fish. Generally, for both non-parasitized and parasitized fish, Cd, Cr and Zn partitioned in the cytosolic fractions and Cu in the particulate fraction. Metal concentrations in organelles within the particulate fractions of the non-parasitized fish were statistically similar except for Cd in the lysosome, while in the parasitized fish, Cd, Cr and Zn were accumulated more by the lysosome and microsomes. In the cytosolic fractions, the non-parasitized fish accumulated Cd, Cr and Zn in the heat stable proteins (HSP), while in the parasitized fish the metals were accumulated in the heat denatured proteins (HDP). On the contrary, Cu accumulated in the HSP in parasitized fish. The present study revealed specific binding of metals to potentially sensitive sub-cellular fractions in fish in the presence of parasites, suggesting interference with metal detoxification, and potentially affecting the health status of fish hosts in Lake Victoria.

1. Introduction

Metals are introduced into aquatic ecosystems through various media such as industrial effluents and wastes, agricultural runoff, domestic garbage dumps and mining activities (Merian, 1991). Increased discharge of metals into aquatic ecosystems may expose aquatic organisms to elevated levels of these metals. Fish have evolved physiological mechanisms, which permit regulation of metals in the body tissues by limiting the uptake or active elimination of excess metals (Giguère et al., 2006), but at high external concentrations, fish may take up more metals than the body can regulate resulting in accumulation and deposition of metals in tissues. The gills and the gastro-intestinal tract are the two main sites for metal uptake in both freshwater and marine fish (Zhang and Wang, 2007). While gills are the main uptake routes for waterborne metals, the gastro-intestinal tract plays a more important role in the uptake of dietary metals (Xu and Wang, 2002).

Fish however, serve as hosts to a range of parasites, which may influence their physiology (Poulin, 1998), potentially affecting their metal regulation capacity (Barber et al., 2000; Kelly and Janz, 2008; Minguez et al., 2009). Modified metal partitioning in fish due to the influence of parasites on the accumulation of metals in various tissues and organs in fish and competition for some specific essential metals with the fish host has indeed been reported (Sures et al., 2003; Oyoo-Okoth et al., 2010a). When in excess in living cells, metals can bind to sensitive target molecules like glutathione, metalloenzymes, DNA or RNA or organelles (nuclei, mitochondria, endoplasmic reticulum and lysosomes) (Wallace et al., 2003). Subcellular partitioning of metals has been investigated in a number of fish species (Olsen and Hogstrand, 1987; Kamunde, 2009; Van Campenhout et al., 2010; Sappal et al., 2009; Liao et al., 2010), but none of these studies have investigated the influence of parasites on the subcellular partitioning of metals in their host. Mechanistic understanding of metal accumulation patterns in parasite–host assemblages may benefit from knowledge of the partitioning of metals in the subcellular fractions. The aim of this study was therefore to determine the metal concentrations in the guts of a cyprinid fish (*Rastrineobola argentea*) parasitized by a cestode, *Ligula intestinalis*, collected from Lake Victoria, Kenya and to

* Corresponding author at: Division of Environmental Health, School of Environmental Studies, Moi University, P.O. Box 3900, Eldoret, Kenya. Tel.: +254 720222082.
E-mail address: elijaoyoo2009@gmail.com (E. Oyoo-Okoth).

0166-445X/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquatox.2011.10.014

quantify the sub-cellular metal distributions in organelles and cytosolic fractions.

Lake Victoria is the second largest fresh water lake in the world and the largest lake in the tropics. It exemplifies a widespread trend of population increase and intensified land-use that has led to increased metal concentrations in the environment. Previous studies have reported elevated levels of Cd, Cr, Cu and Zn in the coastal waters of Lake Victoria (Wandiga, 1981), bottom sediments (Onyari and Wandiga, 1989; Mwamburi, 2003) and in fish (Birungi et al., 2007; Oyoo-Okoth et al., 2010a,b). The cyprinid fish *R. argentea* is one of the three productive fish species in this lake and contains substantial concentrations of metals (Oyoo-Okoth et al., 2010a,b,c). The populations of *R. argentea* in Lake Victoria are further compromised by a very high degree of infestation by the tapeworm *L. intestinalis* (Cowx et al., 2008) that reside in the gut and abdominal cavity of the fish.

2. Materials and methods

2.1. Study area and sampling sites

Lake Victoria (Fig. 1), with an area of 68,800 km^2, is generally shallow (mean depth 40 m) and lies in a catchment of about 184,000 km^2, shared by four riparian states (Kenya, Tanzania, Uganda and Rwanda). Site selection in Kenya was based on the anthropogenic activity profiles along the coastal zones. Site 1 (Kisumu City) has a population of about 1.9 million and is a center of urban development with various industries and drainage of intense agriculture. Site 2 (Kendu-Bay) is a rural area without fertilizer inputs. Site 3 (Karungu) receives drainage from small gold mines. Site 4 (Port Victoria) is a rural area, receiving water from the River Nzoia, containing effluents of two sugar factories, a paper mill factory situated about 100–150 km away from the Lake, and four major urban centers Eldoret, Kitale, Kakamega and Bungoma, with a population of about 1.2 million.

2.2. Water and sediment sampling

Prior to sampling, Van Dorn bottles used for sampling were rinsed with Suprapur HNO_3 and deionized water. A total of 12 water samples per site were taken about 2 m below the surface, using a 3 L Van Dorn bottle. The water samples were then transferred into half-litre polyethylene bottles pre-soaked in nitric and sulphuric acid solutions of 1:1 volume ratio, washed in 2 L of double distilled water and rinsed three times in ultra pure water and dried prior to the field work. The water samples were placed in cool boxes and transported to the laboratory for metal analyses.

A total of 17 sediment samples were collected from each of the four sites. Sediments were collected at the bottom of the lake using an Ekman Grab Sampler. A polypropylene spatula was used to transfer the sediment sub-samples into acid rinsed polypropylene bottles, which were placed in an icebox and transported to the laboratory for metal analyses.

2.3. Fish and parasite collection

The data of the fish and parasite samples is presented in Table 1. A total of 1588 fish samples were obtained on two sampling occasions in March and August 2009 from the sites S1, S2, S3 and S4, using a beam trawl with 5-mm stretched mesh. Fish were attracted in the night by luminescence and captured at the water surface. The collected fish were divided into three sub-samples: one set for whole body metal analyses, the second for gut metal analyses and the last part for subcellular fractions analysis. Fish were rinsed (ultrapure water), weighed (to the nearest 0.1 g) and measured (folk length in mm) and carefully dissected using stainless steel instruments. The whole guts were collected after fish dissection and the entire stomach content emptied. Parasites were removed, counted, stored in glass vials and later transferred into Teflon vials. Parasites, parasitized fish (from which the parasites were removed) and non-parasitized fish were frozen and bagged in ice cool boxes at 0 °C and immediately transported to the laboratory for metal analysis in the International Livestock Research Institute (ILRI, Kenya) laboratory using metal-free techniques.

2.4. Sub-cellular fractionation

To investigate the subcellular partitioning of metals in the gut fractions, guts from 15 individual fish per site were pooled. A total of 20 pooled samples were analyzed per site. To avoid disruption of sub-cellular organelles, the whole digestive tissues were manually homogenized on ice (12 turns of pestle) in a glass cylinder (Glas-Col® product; Tere Haute, IN, USA). Homogenization was performed with 10 mM Tris–HCl buffer (OmniPur, EM Science, affiliate of MERCK KGaA), 0.25 M sucrose with 1 mM phenylmethylsulfonylfluoride (PMSF, as protease inhibitor) and 5 mM dithiothreitol (DTT, as reducing agent), and adjusted to pH 7.2 at 4 °C. The homogenized gut sample was then weighed to determine the wet weight and 0.200 g removed for the determination of the metal concentrations in the total gut. Approximately 4.500 g of the sub-sample of gut homogenate was used for the determination of sub-cellular partitioning using a differential centrifugation protocol adapted from Campbell et al. (2005) and Raimundo et al. (2008). The gut homogenate was first fractioned by centrifugation at 700 × *g* for 15 min at 4 °C to separate the nuclei; the supernatant was further centrifuged at 9000 × *g* for 20 min at 4 °C to collect the mitochondrial fraction; the lysosome and microsomal fractions were obtained by centrifuging the supernatant at 30,000 × *g* for 25 min, and 100,000 × *g* for 40 min at 4 °C, respectively. To separate the cytosolic fractions, a procedure adopted from Wallace et al. (2003) and Wallace and Luoma (2003) was applied. After centrifugation at 100,000 × *g* for 60 min and weighing the contents, the homogenate was further centrifuged at 50,000 × *g* for 10 min in order to obtain a pool of heat denaturable protein (HDP) and a final soluble supernant of heat stable protein (HSP). The subcellular fractions obtained by the centrifugation procedure were stored at −20 °C until analyzed for metal concentrations.

2.5. Metal analysis

All samples were separately weighed and then subjected to acid digestion using an Ethos D (Type Ethos plus 1) microwave lab station (Monroe, CT, USA) prior to analysis. For each sample, a maximum of 0.200 g was weighed and placed in a Teflon vessel with 7.0 mL of concentrated nitric acid (65%), 1.0 mL concentrated hydrochloric acid (30%) and 1.0 mL hydrogen peroxide (30%). The samples were then placed in the microwave oven and subjected to a microwave program as follows: Step 1: 25–200 °C for 10 min at 1000 W; Step 2: 200 °C for 10 min at 1000 W. Digests were finally made up with ultra pure de-ionised water to 25.0 mL in acid washed standard flasks. Finally, the diluted solutions were transferred into acid cleaned polyethylene bottles. Thermo electron X7 inductively coupled plasma mass spectrometry (ICP-MS), model X series, UK was used to analyze the water and the digested samples for the concentrations of Cd, Cr, Zn and Cu. Calibration standards were purchased from High-Purity Standards (Charleston, SC 29423, USA). The standards were appropriately diluted and used to calibrate the ICP-MS before metal determinations in samples. The ICP-MS operating conditions were: nebulizer gas flow 0.91 L/min, radio frequency (RF) 1200 W, lens voltage 1.6 V, cool gas 13.0 L/min, auxiliary gas 0.70 L/min. The detection limits were (in μg/g) 0.02

Table 1
Data of the fish and parasite samples collected from the four sampling sites in Lake Victoria.

Catch data	Sampling sites			
	S1 (Kisumu)	S2 (Kendu Bay)	S3 (Karungu)	S4 (Port Victoria)
Catch date	April–July 2009	March–July 2009	March–August 2009	April–August 2009
Number of fish	400	396	392	400
Sex ratio (M:F)	18:15	7:8	16:21	16:19
Mean length ± SEM of *R. argentea* (mm)	37.0 ± 1.6	44.4 ± 1.4	49.1 ± 1.3	38.4 ± 2.2
Mean weight ± SEM of non parasitized *R. argentea* (g)	1.37 ± 0.17	1.49 ± 0.30	1.75 ± 0.34	1.55 ± 0.42
Mean weight ± SEM of parasitized *R. argentea* (g)	2.26 ± 0.23	2.40 ± 0.30	2.71 ± 0.32	2.37 ± 0.29
Mean weight ± SEM of gut of non parasitized *R. argentea* (g)	0.50 ± 0.03	0.54 ± 0.04	0.55 ± 0.04	0.58 ± 0.06
Mean weight ± SEM of gut of parasitized *R. argentea* (g)	0.48 ± 0.03	0.53 ± 0.03	0.51 ± 0.04	0.58 ± 0.05
Number of *Ligula intestinalis* per fish	1.99 ± 0.15	2.01 ± 0.22	2.34 ± 0.02	1.07 ± 0.07

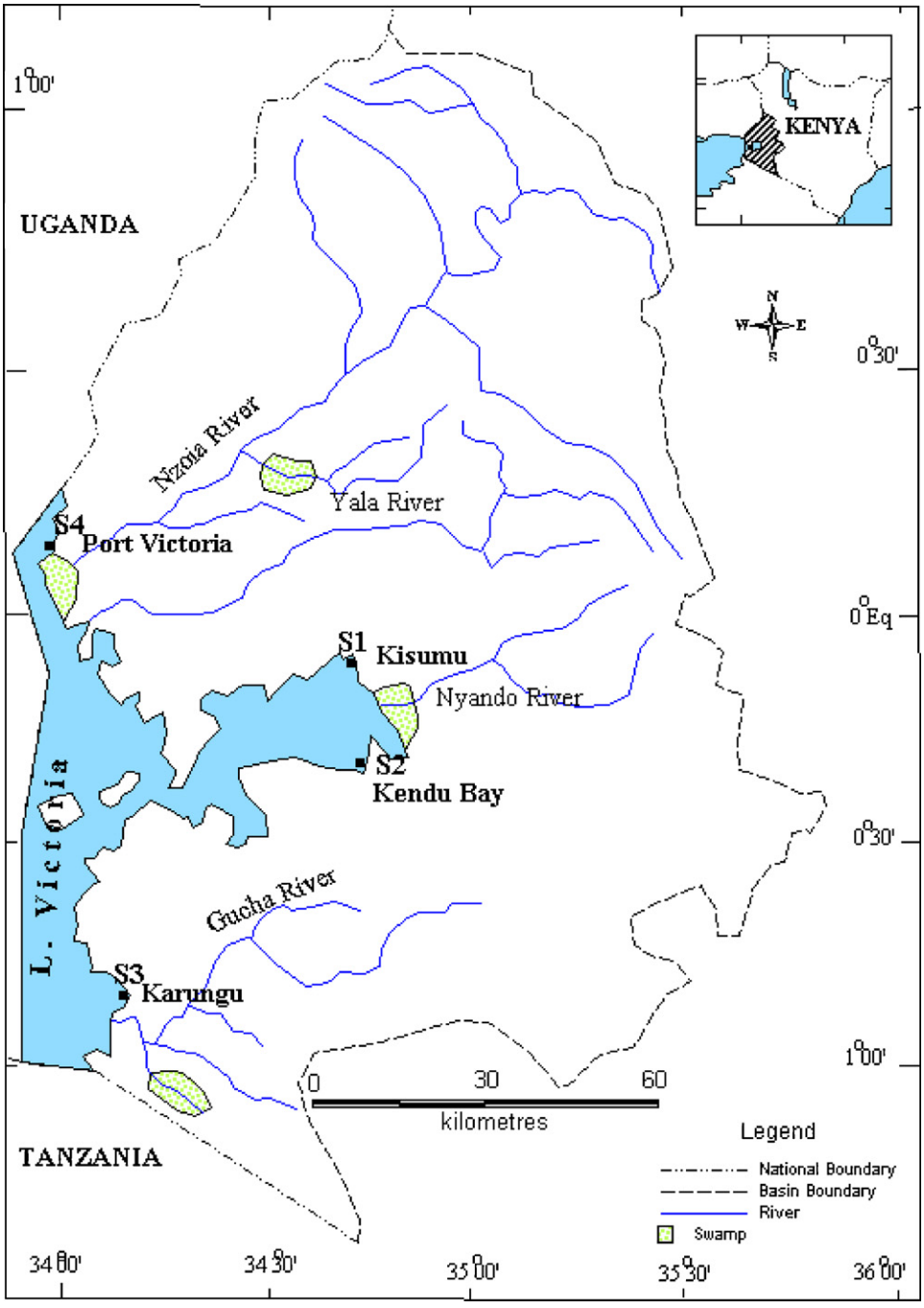

Fig. 1. Sampling site locations in the Lake Victoria Basin, Kenya.

(Cd), 0.02 (Cr), 0.15 (Zn) and 0.04 (Cu). The reference tissues dogfish muscle DORM-2 (NRCC) and lobster hepatopancreas TORT-2 (NRCC) were treated and analyzed in the same way as the samples. The accuracy of the measurement of the certified values of the reference materials (%, $N = 3$) was: Cd 96.4 ± 3.2; Cr 98.1 ± 2.4; Cu 99.3 ± 2.7; Zn 101 ± 2.6. As an internal control on sample manipulation and analysis, element burdens were calculated for each fraction in the differential centrifugation procedure ([X] × weight of fraction; X = Cd, Cr, Zn and Cu) and the sum of the individual burdens was compared with gut burden determined by analysis of a sub-sample of the original gut homogenate. Agreement was generally good for Cd: 102 ± 5%, for Cr: 98 ± 4%, for Zn: 99 ± 4% and for Cu: 100 ± 4% ($N = 17$; mean ± SD).

2.6. Statistical analyses

Metal concentrations in water, sediments, non-parasitized fish, parasitized fish and in the fish parasites among the sampling sites were compared using a one-way ANOVA. Duncan's Multiple Range Test (DMRT) was used for post-hoc discrimination between the means. At each site, differences in metal concentrations between non-parasitized and parasitized fish were determined using one-way ANOVA. For metal concentrations the interaction between site (S1, S2, S3 and S4) and parasitic infestation (parasitized and non-parasitized fish) was analyzed using two-way ANOVA. The interaction between site, parasitic infestation and the subcellular fraction was examined using three-way ANOVA. Linear regression analyses were used to evaluate the relationships between metal concentrations in the gut of the parasitized fish and fish parasites as well as between the subcellular fractions and the whole gut of the fish. Regression parameters were estimated using the ordinary least-squares equation. The *t*-test was used to verify that regression residuals were normally distributed and homoscedasticity was checked by examination of the biplots of residuals against predicted values. The statistical analyses were performed using the STATISTICA 8.0 Statistical Software.

3. Results

3.1. Metal concentrations in water and sediments

The Cd, Cr, Zn and Cu concentrations in water and sediments are shown in Table 2. Metal concentrations in water were generally low, although they exhibited significant (one-way ANOVA test; $p < 0.05$) spatial variations. The highest Cd concentration occurred at site 2, while Cr, Cu and Zn concentrations were highest at site 3. Similar trends in metal concentrations occurred in sediments, containing relatively high concentrations. The Cd concentration was significantly (one-way ANOVA test; $p < 0.05$) higher at site 2 than at the other sites, while Cr, Zn and Cu concentrations were significantly ($p < 0.05$) higher at site 3.

3.2. Metal concentrations in the whole body and in the gut of non-parasitized and parasitized R. argentea

Concentrations of Cd, Cr, Zn and Cu in the whole body of non-parasitized and parasitized *R. argentea*, and in the parasites are shown in Table 3. All metals in non parasitized, parasitized fish and in the parasites (except Cu) displayed significant differences between sites (one-way ANOVA; $p < 0.05$). Cd in fish was elevated at site 1 while Cr, Zn and Cu concentrations were high at site 3. For metal concentrations there were significant interactions between infestation and site (two-way ANOVA; $p < 0.05$). The concentrations of Cd, Cr and Zn were significantly higher in parasitized than in non-parasitized fish at all sites except Zn at sites 2 and 3 (one-way ANOVA; $p < 0.05$), while the Cu concentration was significantly lower in parasitized fish than non parasitized fish at all sites ($p < 0.05$).

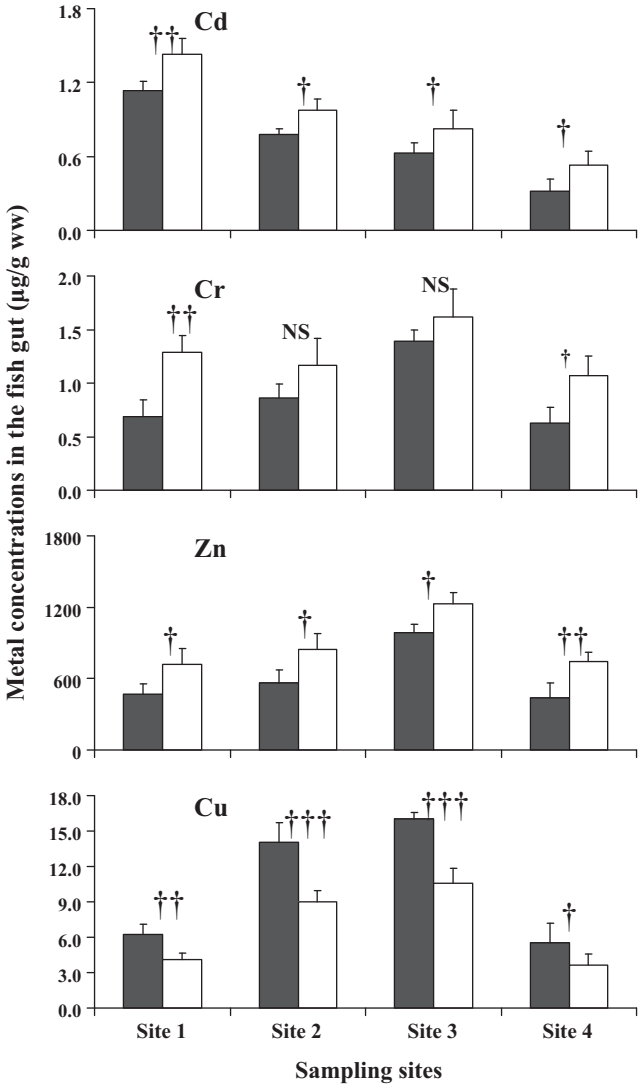

Fig. 2. Concentrations (mean ± SEM; $n = 3$) of Cd, Cr, Zn and Cu (µg/g ww) in the gut of non parasitized fish (black bars) and parasitized fish (white bars) at the four sampling sites in Lake Victoria. Values with †, †† and ††† indicate a significant difference between metal concentrations in the gut of non-parasitized and parasitized fish at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. NS denotes no significant difference.

Metal concentrations in the gut of the non-parasitized and parasitized fish are presented in Fig. 2. In agreement with the results for the whole body, the concentrations of Cd, Cr and Zn in the gut were significantly higher in parasitized fish than in non-parasitized fish at all sites except Zn in sites 2 and 3 (one-way ANOVA; $p < 0.05$), while the Cu concentration was significantly ($p < 0.05$) lower in the gut of parasitized fish than in that of the non-parasitized fish at all sites. There were also site specific differences in the metal accumulation in the gut of non-parasitized and parasitized fish (one-way ANOVA; $p < 0.05$), with Cd being accumulated more at site 1, Cr and Zn at site 3 and Cu at sites 2 and 3.

Metal concentrations in the gut of the fish relative to those in the parasites are shown in Fig. 3. The concentrations of Cd, Cr and Zn in the gut of the parasitized fish were significantly (*t*-test; $p < 0.05$) positively correlated with the respective metals in the parasites. Again in contrast, the Cu concentration in the gut displayed a

Table 2
Metal concentrations (mean ± SEM) in water ($n = 12$) and sediment ($n = 17$) from four sampling sites in Lake Victoria. Values expressed in μg/L for water; μg/g ww for sediments. Values with different letters differ significantly ($p < 0.05$) based on one-way ANOVA test followed by post-hoc Duncan's Multiple Range Test.

Metal concentration	Sampling sites				*F*	*p*-value
	S1	S2	S3	S4		
Water						
Cd	0.04 ± 0.01^{a}	0.08 ± 0.01^{b}	0.05 ± 0.01^{a}	0.04 ± 0.01^{a}	8.183	0.0043
Cr	0.15 ± 0.04^{a}	0.22 ± 0.06^{b}	0.51 ± 0.09^{d}	0.31 ± 0.06^{c}	83.361	0.0000
Zn	35.4 ± 11.9^{a}	36.3 ± 16.2^{a}	71.2 ± 15.2^{c}	56.3 ± 14.2^{b}	52.421	0.0000
Cu	11.2 ± 3.7^{a}	10.4 ± 2.3^{a}	26.5 ± 12.2^{c}	17.4 ± 4.9^{b}	52.459	0.0000
Sediment						
Cd	5.5 ± 0.7^{a}	6.9 ± 0.09^{b}	5.8 ± 0.08^{a}	5.2 ± 0.7^{a}	13.113	0.0023
Cr	2.2 ± 0.1^{a}	3.5 ± 0.7^{b}	8.4 ± 1.1^{d}	6.2 ± 0.9^{c}	81.329	0.0000
Zn	119.3 ± 23.4^{a}	195.5 ± 24.1^{b}	259 ± 23.4^{c}	122.1 ± 27.3^{a}	56.623	0.0000
Cu	20.1 ± 4.6^{a}	32.0 ± 3.7^{b}	57.0 ± 6.1^{d}	50.0 ± 6.8^{c}	78.419	0.0000

significant (*t*-test; $p < 0.05$) negative correlation with the Cu concentration in the parasites.

3.3. Metal concentrations in the gut and subcellular fractions of non-parasitized and parasitized R. argentea

The metal concentrations in the particulate and cytosolic fractions of non-parasitized and parasitized fish from the four sampling sites in Lake Victoria are shown in Fig. 4 while the associated statistical analyses of the interactions are reported in Table 4. By comparing the metal content in the particulate fractions (nuclei + mitochondria + lysosomes + microsomes) and in the cytosole (HDP + HSP), it was estimated that significantly ($p < 0.05$) higher concentrations of Cd (67.2%), Cr (69.4%), and Zn (64.2%) were present in the cytosolic fraction in the non-parasitized fish compared to the parasitized fish (Cd, 56.1%; Cr, 58.5%; Zn, 54.8%). On the contrary, the particulate Cu concentration in the non-parasitized fish (31.5%) was significantly (one-way ANOVA; $p < 0.05$) higher than in the parasitized fish (23.6%). For metal concentrations in both the non-parasitized and parasitized fish, there were significant interactions between site and subcellular fraction (two-way ANOVA; $p < 0.05$). The interactions were also significant between site and infestation (two-way ANOVA; $p < 0.05$). The three-way ANOVA indicated that there were also significant interaction between site, subcellular fraction and infestation ($p < 0.05$). Although there were significant differences in the concentrations of Cr and Zn in the four particulate fractions at the four sites (two-way ANOVA; $p < 0.05$) for the non-parasitized fish, the differences were not large. However, the concentration of Cd was significantly higher in the lysosomes of non-parasitized fish than in the other organelles at all the sites (two-way ANOVA; $p < 0.05$), while at all sites Cu in the nuclei was significantly higher (two-way ANOVA; $p < 0.05$) than in the other particulate fractions in the non-parasitized fish. On the contrary, in the parasitized fish, the lysosomes and microsomes accumulated significantly (one-way ANOVA; $p < 0.05$) higher amounts of Cd, Cr and Zn than the other organelles, while the mitochondria accumulated significantly higher (one-way ANOVA; $p < 0.05$) concentrations of Cu. Concerning the cytosolic fraction, the non-parasitized fish contained significantly higher concentrations of Cd, Cr and Zn in the heat stable proteins (HSP) than in heat denatured proteins (HDP) at all sampling sites (two-way ANOVA; $p < 0.05$), while in the parasitized fish, Cd, Cr and Zn accumulated more in the HDP at all sampling sites (two-way ANOVA; $p < 0.05$). Cu concentrations in the

Table 3
Metal concentrations (μg/g ww; mean ± SE) in the whole body of non parasitized fish, parasitized fish and in the parasites at the four sampling sites in Lake Victoria. Numbers of samples for non parasitized fish: Site 1, $n = 28$; Site 2, $n = 28$; Site 3, $n = 31$; Site 4, $n = 28$; for the parasitized fish: S1, $n = 29$; S2, $n = 26$; S3, $n = 31$; S4, $n = 31$; and for parasites: S1, $n = 29$; S2, $n = 27$; S3, $n = 31$; S4, $n = 31$. Values with different letters differ significantly ($p < 0.05$) among the sampling sites (one-way ANOVA followed by post-hoc Duncan's Multiple Range Test). NS denotes no significant difference for that specific element. *F* values were calculated by ANOVA; *p*-values for the same analysis are in parenthesis.

Metal	Sampling sites				*F*	*p*-value
	S1	S2	S3	S4		
Non parasitized fish						
Cd	0.24 ± 0.06^{b}	0.19 ± 0.05^{a}	$0.21 \pm 0.05^{a,b}$	0.19 ± 0.08^{a}	33.183	0.0000
Cr	0.49 ± 0.12^{a}	0.58 ± 0.13^{b}	0.62 ± 0.14^{b}	0.45 ± 0.13^{a}	23.375	0.0000
Zn	180.6 ± 23.8^{a}	185.2 ± 49.6^{a}	261.9 ± 35.4^{b}	$214.5 \pm 47.8^{a,b}$	120.888	0.0000
Cu	7.42 ± 0.81^{f}	6.16 ± 0.51^{e}	10.38 ± 1.35^{g}	5.74 ± 0.99^{e}	33.415	0.0000
Parasitized fish						
Cd	0.53 ± 0.08^{d}	0.42 ± 0.06^{c}	0.44 ± 0.07^{c}	0.41 ± 0.04^{c}	16.438	0.0001
Cr	1.09 ± 0.11^{c}	1.01 ± 0.04^{c}	1.15 ± 0.04^{d}	0.93 ± 0.16^{c}	12.645	0.0002
Zn	196.7 ± 14.9^{c}	206.4 ± 19.6^{c}	277.2 ± 26.6^{d}	268.6 ± 27.8^{d}	22.376	0.0000
Cu	2.86 ± 0.23^{b}	2.09 ± 0.32^{a}	4.18 ± 0.42^{d}	3.35 ± 0.32^{c}	15.406	0.0001
Main effects (between parasitized and non parasitized fish)					Interaction (Site × Parasite infestation)	
Cd	506.216(0.0000)	172.165(0.0000)	136.223(0.0000)	117.275(0.0000)	11.277	0.0003
Cr	130.755(0.0000)	22.698(0.0001)	5.994(0.0204)	35.872(0.0000)	7.355	0.0009
Zn	5.818(0.0000)	3.828(0.059)	1.993(0.1673)	78.124(0.0000)	62.309	0.0000
Cu	809.777(0.0000)	922.144(0.0000)	350.607(0.0000)	360.082(0.000)	23.419	0.0000
Fish parasites						
Cd	0.74 ± 0.12^{b}	0.75 ± 0.16^{b}	0.69 ± 0.17^{b}	0.54 ± 0.11^{a}	17.146	0.0001
Cr	1.74 ± 0.28^{b}	1.31 ± 0.24^{a}	1.67 ± 0.25^{b}	1.34 ± 0.19^{a}	2.864	0.0041
Zn	117.6 ± 30.6^{a}	216.2 ± 70.4^{b}	343.7 ± 61.3^{c}	256.6 ± 45.8^{b}	53.281	0.0000
Cu	15.31 ± 3.43	15.16 ± 4.97	14.07 ± 3.69	15.02 ± 5.24^{NS}	2.221	0.0812

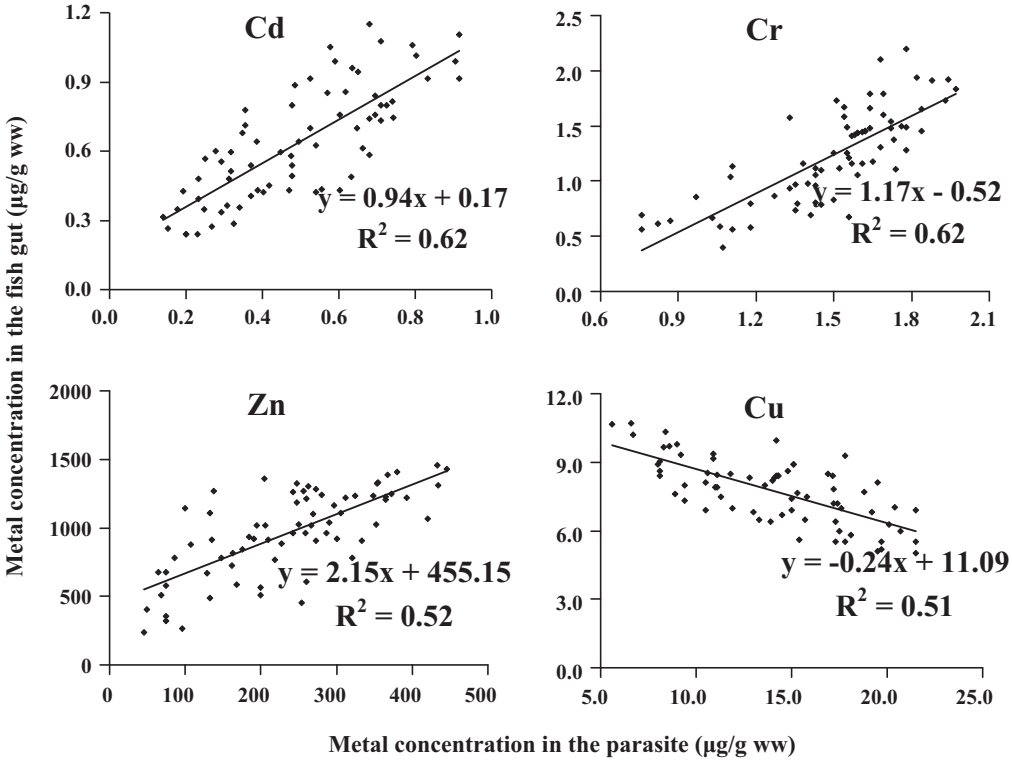

Fig. 3. Relationships between the Cd, Cr, Zn and Cu concentrations in the gut of parasitized fish (µg/g ww) and in the parasites (µg/g ww) from the four sampling sites in Lake Victoria. Numbers of samples: S1, $n = 29$; S2, $n = 26$; S3, $n = 31$; S4, $n = 31$.

cytosolic fraction were similar between HSP and HDP in the non-parasitized fish at all sites (two-way ANOVA; $p > 0.05$), but were significantly higher in the HSP fractions in the parasitized fish at all sites (two-way ANOVA; $p < 0.05$).

Relationships between metal concentrations in the total gut and in the subcellular fractions of parasitized and non-parasitized fish are shown in Fig. 5. In non-parasitized fish, the concentrations of Cd, Cr and Zn in the particulate fractions were significantly ($R^2 > 0.5$; *t*-test; $p < 0.05$;) positively correlated with those in the gut, albeit the slope was much lower than in the case of parasitized fish ($R^2 < 0.3$; although $p < 0.05$, *t*-test). However, the Cu concentrations in all organelles in the particulate fraction of parasitized fish were significantly (*t*-test; $p < 0.05$) negatively correlated to those in the gut; although for mitochondria this was not significant (*t*-test; $p > 0.05$).

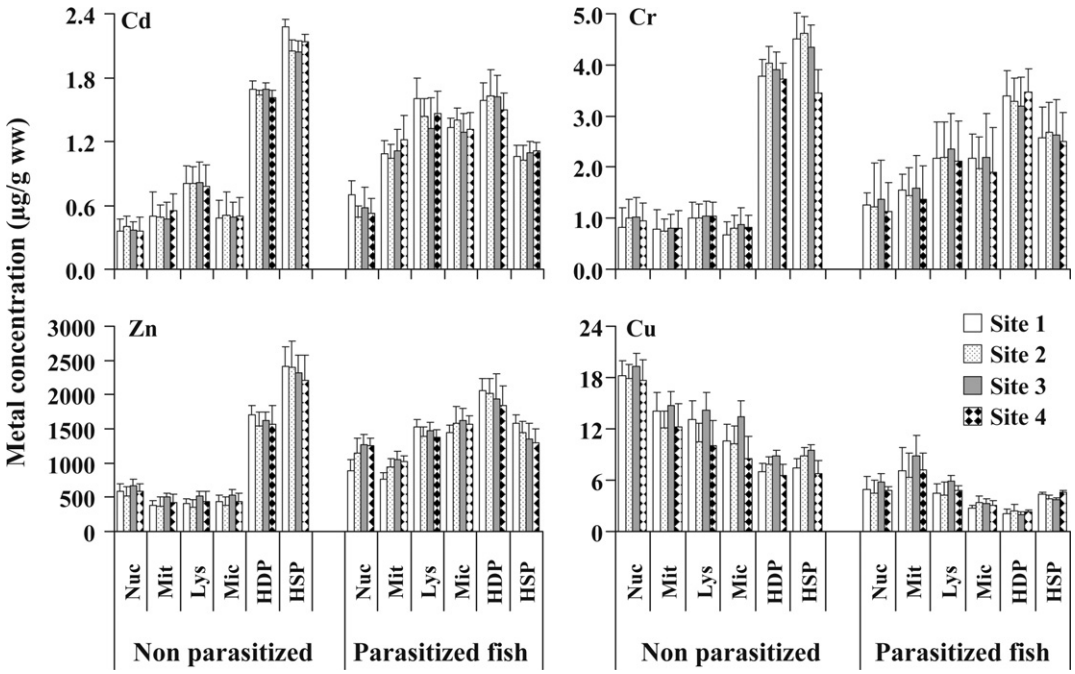

Fig. 4. Cd, Cr, Zn and Cu concentrations (mean ± SEM; $n = 3$) in the particulate (nuclei, Nuc; mitochondria, Mit; lysosomes, Lys; microsomes, Mic) and cytosolic (heat denaturable protein, HDP and heat stable protein, HSP) fractions in non-parasitized and parasitized fish (µg/g ww) from the four sampling sites in Lake Victoria.

Table 4
Two-way and three-way analyses of variance (ANOVA) of metal concentrations in the organelles showing interactions between site (S1, S2, S3 and S4), subcellular fraction (particulate fractions: nuclei, mitochondria, lysosomes and ribosomes; cytosolic fractions: heat denatured protein and heat stable proteins) and fish infestation (non parasitized and parasitized fish).

Fraction	Metal			*F*	*p*-value
Particulate	Cd	2 way interaction	Site × Subcellular fraction (Non parasitized fish)	19.140	0.0000
			Site × Subcellular fraction (Parasitized fish	124.186	0.0000
			Site × Parasitic infestation	90.371	0.0000
		3 way interaction	Site × Parasitic infestation × Subcellular fraction	20.634	0.0000
	Cr	2 way interaction	Site × Subcellular fraction (Non parasitized fish)	6.895	0.0001
			Site × Subcellular fraction (Parasitized fish)	39.576	0.0000
			Site × Parasitic infestation	115.892	0.0000
		3 way interaction	Site × Parasitic infestation × Subcellular fraction	91.123	0.0000
	Zn	2 way interaction	Site × Subcellular fraction (Non parasitized fish)	14.318	0.0000
			Site × Subcellular fraction (Parasitized fish)	84.631	0.0000
			Site × Parasitic infestation	13.112	0.0000
		3 way interaction	Site × Parasitic infestation × Subcellular fraction	51.324	0.0000
	Cu	2 way interaction	Site × Subcellular fraction (Non parasitized fish)	11.508	0.0000
			Site × Subcellular fraction (Parasitized fish)	22.737	0.0000
			Site × Parasitic infestation	149.948	0.0000
		3 way interaction	Site × Parasitic infestation × Subcellular fraction	21.324	0.0000
Cytosolic	Cd	2 way interaction	Site × Subcellular fraction (Non parasitized fish)	9.254	0.0000
			Site × Subcellular fraction (Parasitized fish)	15.620	0.0000
			Site × Parasitic infestation	38.557	0.0000
		3 way interaction	Site × Parasitic infestation × Subcellular fraction	32.279	0.0000
	Cr	2 way interaction	Site × Subcellular fraction (Non parasitized fish)	15.464	0.0000
			Site × Subcellular fraction (Parasitized fish)	27.868	0.0000
			Site × Parasitic infestation	28.744	0.0000
		3 way interaction	Site × Parasitic infestation × Subcellular fraction	21.512	0.0000
	Zn	2 way interaction	Site × Subcellular fraction (Non parasitized fish)	6.471	0.0002
			Site × Subcellular fraction (Parasitized fish)	29.702	0.0000
			Site × Parasitic infestation	17.487	0.0000
		3 way interaction	Site × Parasitic infestation × Subcellular fraction	5.122	0.0003
	Cu	2 way interaction	Site × Subcellular fraction (Non parasitized fish)	4.427	0.0006
			Site × Subcellular fraction (Parasitized fish)	29.122	0.0000
			Site × Parasitic infestation	88.186	0.0000
		3 way interaction	Site × Parasitic infestation × Subcellular fraction	44.718	0.0000

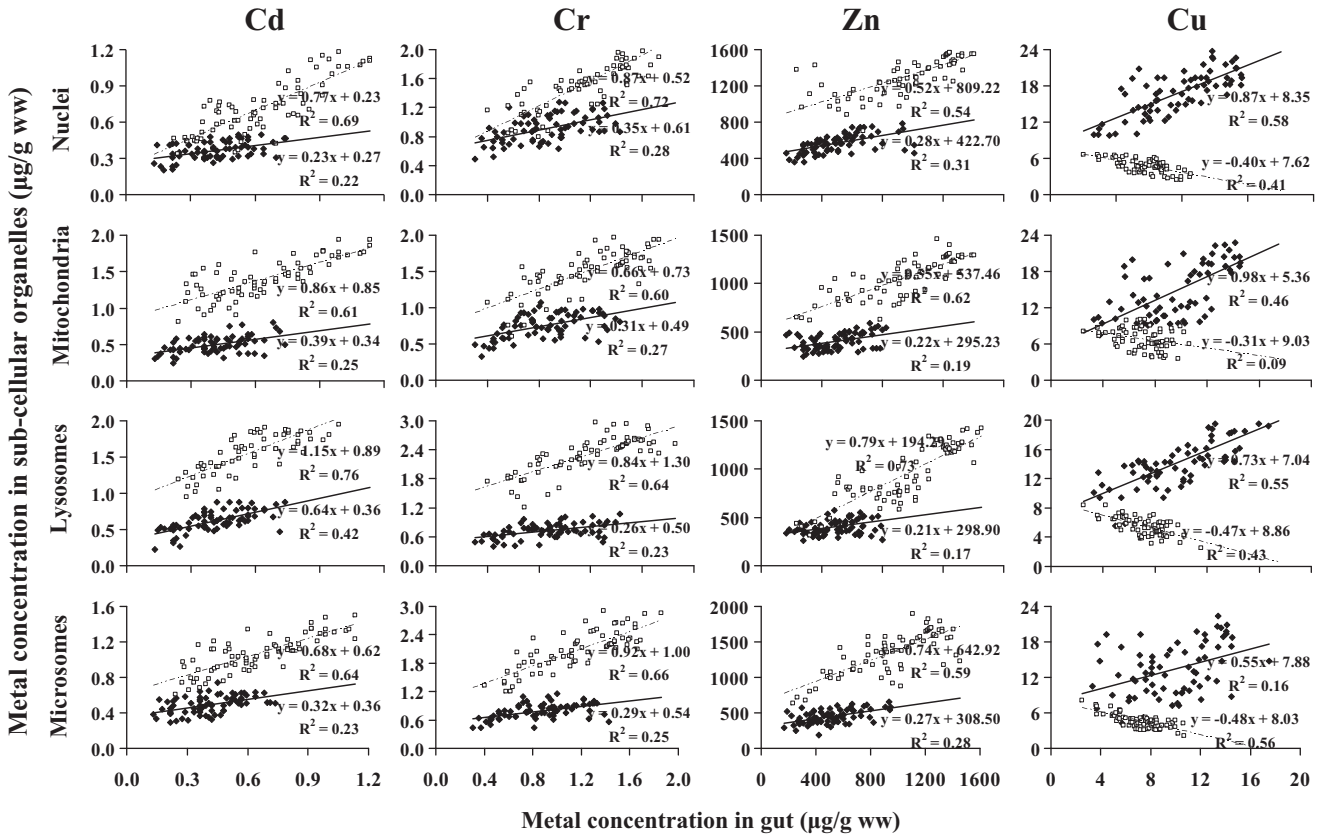

Fig. 5. Relationships between the Cd, Cr, Zn and Cu concentrations (µg/g ww) in nuclei, mitochondria, lysosomes and microsomes and those in the gut of non parasitized fish (black diamonds) and parasitized fish (white squares) from the four sampling sites in Lake Victoria.

The relationships between metal concentrations in the cytosolic fractions and in the gut were not significant (t-test; $p > 0.05$) (data not shown).

4. Discussion

The digestive gland of fish is the key organ for metabolism, vital for dietary metal uptake and excretion. Both non-essential and essential metals in excess of physiological needs must either be excreted or stored in an insoluble form to prevent accumulation in tissues where they may cause harm. The present study demonstrated that this regulatory mechanism in fish is disrupted by parasites. The patterns of metal accumulation in the whole body and in the gut differed between non-parasitized and parasitized fish. The parasitized fish accumulated more Cd, Cr and Zn in the whole body and in gut than the non-parasitized fish with clear positive correlations between metals in the gut and in the parasites, consistent with our previous study on metal accumulation in the whole body of this fish (Oyoo-Okoth et al., 2010a) and with metal accumulation in *L. intestinalis* in tench (*Tinca tinca*) (Tekin-Özan and Kir, 2005), roach (*Rutilus rutilus*) and silver bream (*Blicca bjorkna*) (Tenora et al., 2000).

The interaction between fish gut and parasites in the uptake of metals is not well understood. However, recently it was demonstrated that cestode parasites absorb numerous protein, lipids and an unknown quantity of other substances from the hosts (Jawale et al., 2011). Therefore we hypothesize that in addition to the proteins required for metabolic needs, parasites may also absorb metal binding proteins from the hosts and therefore interfere with the metal detoxification role of these metallo-proteins in the host and this may explain the higher Cd, Cr and Zn concentrations in the parasitized fish compared to the non-parasitized fish.

Parasites may also interfere with uptake and regulation of essential metals (Poulin, 1998). Interference with metal uptake in the hosts has previously been demonstrated in the *Pomphorhynchus laevis* and *Leuciscus cephalus* assemblage (Sures et al., 2003). The lower Cu concentrations in the body of parasitized fish together with the negative correlation between the Cu concentration in the fish body and in the parasite suggests that parasites competed for copper with the fish host, as was previously observed by Sures (2002) and Oyoo-Okoth et al. (2010a).

In the particulate fractions (nuclei, mitochondria, lysosome and microsomes) of the gut in non-parasitized fish, Cd was accumulated more in the lysosomes than in the other organelles. Lysosomes accumulate metals from the cytosolic fractions of the digestive gland cells for eventual elimination (Bustamante et al., 2002) and may therefore signify the potential elimination pathway for Cd in the non-parasitized fish. In contrast, the high accumulation of Cd, Cr and Zn in the particulate fractions of the parasitized fish may indicate binding of these metals to metal sensitive sites. Likewise, the stronger correlation between Cd, Cr and Zn concentrations in the particulate fractions and the whole gut of parasitized fish suggested higher metal uptake rates in the organelles, likely associated with compromised physiological metal regulation in parasitized fish. Cu however, accumulated more in the particulate fractions of the non-parasitized fish and appeared to be regularly absorbed from the gut in non-parasitized fish. In contrast to Cd, Cr and Zn, the Cu concentration in the particulate fractions of the parasitized fish was negatively correlated with the Cu concentration in the whole gut, suggesting Cu sequestration by parasites, as reported for the partitioning of Cu between the whole body of parasitized *R. argentea* and *L. intestinalis* (Oyoo-Okoth et al., 2010a). The low metal concentration, uniform distribution and low rate of increase in particulate fraction of the gut of non-parasitized fish, indicated that healthy fish was sufficiently capable of detoxifying the non-essential metals. Yet when parasitized, these mechanisms were disrupted, leading to high accumulation of metals in the particulate fractions and higher metal uptake rates from the gut.

The cytosolic fraction in the non-parasitized *R. argentea* contained a high proportion of Cd, Cr, and Zn in the heat stable protein component. This is in agreement with previous studies (Olsson and Haux, 1986; Dallinger et al., 1997; Campbell et al., 2005, 2008; Kraemer et al., 2005). The HSP contain metal binding ligands (e.g., metallothionein or metallothionein-like proteins and glutathione), which are heat stable and can chelate and/or sequester metals by intercepting their ions as they are taken up and trafficked through cellular compartments or abstract them from metal-sensitive biomolecules and organelles (Kamunde, 2009). However, in the parasitized fish, Cd, Cr, and Zn partitioned in the HDP fraction. The HDP is assumed to correspond largely to denatured enzymes and other non-enzymatic proteins (e.g., albumin and haemoglobin) (Giguère et al., 2006). The presence of Cd, Cr and Zn in high concentration in this fraction due to the presence of parasites reflects that toxic metals were bound to metal sensitive sites. In contrast to Cd, Cr and Zn, the concentration of Cu in the cystolic fraction of the gut of *R. argentea* was low in the parasitized fish, suggesting that metallothioneins may not play a major role in Cu regulation. The low Cu accumulation in the cytosolic fraction of the parasitized fish and the negative correlation between Cu concentrations in fish and parasites could indicate that the parasite competed for Cu with the fish and therefore sequester it from the metal binding sites. Although the role of the cytosole was reduced in Cu uptake in parasitized fish, a significant accumulation in the potentially metal sensitive fractions notably HDP, was observed which points to either increased uptake through the HDP or a possible imperfect regulation in parasitized fish.

Until recently, concerns have been expressed over the increased prevalence of *L. intestinalis* in *R. argentea* (Marshall and Cowx, 2003; Cowx et al., 2008), which may have serious implications for the Lake Victoria fishery, because of the potential of the cestode to cause fish population collapses (Wyatt and Kennedy, 1988, 1989). Recent studies have indicated that infections of *R. argentea* by *L. intestinalis* resulted in late maturation, reduced fecundity and possible increased stress by acting on the brain-pituitary-gonadal axis (Cowx et al., 2008). This study adds further to the complexity of the parasitic infections of *R. argentea* by *L. intestinalis*. The specific binding of Cd and Cr, considered non-essential metals, to metal sensitive sites in the parasitized fish could point toward toxicity to *R. argentea*, while the depletion of Cu in parasitized *R. argentea* may lead to nutritional deficiencies. If these indications and interpretations are correct then there are serious implications for the viability of the Lake Victoria fishery on *R. argentea*.

5. Conclusions

This study showed that parasitized fish accumulated metals in the whole body, the gut and subcellular fractions in metal specific patterns differing from those in non-parasitized fish. The changes in the partitioning of metals showed that parasites deregulate uptake and elimination of toxic metals by fish, and competed for Cu with the fish. The present study revealed specific binding of metals to potentially sensitive sub-cellular fractions in fish in presence of parasites, suggesting interference with metal detoxification at the cellular levels, with serious implications for the health of fish hosts and Lake Victoria *R. argentea* fishery.

Acknowledgements

This study was supported by Government of The Netherlands, through the NWO-WOTRO funding scheme (Grant no. W

01.83.2004.023). We are very grateful for the assistance of Mr. Kuloba of ILRI for metal analysis and Tonny Odero of Kenya Marine and Fisheries Research Institute (KEMFRI) for their assistance in the sampling and a laboratory for sample preparation. We also thank the local fishermen in Kenya who provided their boats and assistance during the sampling time in Lake Victoria.

References

Barber, I., Hoare, D., Krause, J., 2000. Effects of parasites on fish behaviour: a review and evolutionary perspective. Rev. Fish Biol. Fish 10, 131–165.

Birungi, Z., Masola, B., Zaranyika, M.F., Naigaga, I., Marshall, B., 2007. Active biomonitoring of trace heavy metals using fish of trace heavy metals using fish (*Oreochromis niloticus*) as bioindicator species. The case of Nakivubo wetland along Lake Victoria. Phys. Chem. Earth 15-18, 1350–1358.

Bustamante, P., Cosson, R., Gallien, I., Caurant, F., Miramand, P., 2002. Cadmium detoxification processes in the digestive gland of cephalopods in relation to accumulated cadmium concentrations. Mar. Environ. Res. 53, 227–241.

Campbell, P.G.C., Giguère, A., Bonneris, E., Hare, L., 2005. Cadmium-handling strategies in the chronically exposed indigenous freshwater organisms – the yellow perch (*Perca flavescens*) and the floater mollusc (*Pyganodon grandis*). Aquat. Toxicol. 72, 73–97.

Campbell, P.G.C., Kraemer, L.D., Giguére, A., Hare, L., Hontela, A., 2008. Subcellular distribution of cadmium and nickel in chronically exposed wild fish: inferences regarding metal detoxification strategies and implications for setting water quality guidelines for dissolved metals. Hum. Ecol. Risk. Assess. 14, 290–316.

Cowx, I.G., Rollins, D., Tumwebaze, R., 2008. Effects of *Ligula intestinalis* on the reproductive capacity of *Rastrineobola argentea* in Lake Victoria. J. Fish Biol. 73, 2249–2260.

Dallinger, R., Egg, M., Kock, G., Hofer, R., 1997. The role of metallothionein in cadmium accumulation of Arctic char (*Salvelinus alpinus*) from high alpine lakes. Aquat. Toxicol. 38, 47–66.

Giguère, A., Campbell, P.G.C., Hare, L., Couture, P., 2006. Subcellular partitioning of cadmium, copper, nickel and zinc in indigenous yellow perch (*Perca flavescens*) sampled along a polymetallic gradient. Aquat. Toxicol. 77, 178–189.

Jawale, S., Fartade, A., Borde, S., 2011. Biochemical studies of *Caryophyllidean* tapeworms in fresh water fish *Clarias batrachus*. Rec Res. Sci. Tech. 3, 35–36.

Kamunde, C., 2009. Early subcellular partitioning of cadmium in the gills and liver of rainbow trout (*Onchorhynchus mykiss*) following low-to-near-lethal waterborne cadmium exposure. Aquat. Toxicol. 91, 291–301.

Kelly, J.M., Janz, D., 2008. Altered energetics and parasitism in juvenile northern pike (*Esox lucius*) inhabiting metal-mining contaminated lakes. Ecotoxicol. Environ. Saf. 70, 357–369.

Kraemer, L.D., Campbell, P.G.C., Landis, H., 2005. Dynamic of Cd, Cu and Zn accumulation in the organs and subcellular fractions in field transplanted juvenile yellow perch (*Perca flavescens*). Environ. Pollut. 142, 313–325.

Liao, C.-M., Ju, Y.-R., Chen, W.-Y., 2010. Subcellular partitioning links BLM-based toxicokinetics for assessing cadmium toxicity to rainbow trout. Environ. Toxicol., doi:10.1002/tox.20584.

Marshall, J., Cowx, I.G., 2003. Will the explosion of *Ligula intestinalis* in *Rastrineobola argentea* lead to another shift in the fisheries of Lake Victoria? In: Cowx, I.G. (Ed.), Interactions Between Fish and Birds: Implications for Management. Fishing News Books, Blackwell Science, Oxford, pp. 244–256.

Merian, E., 1991. Metals and their Compounds in the Environment. Occurrence, Analysis and Biological Relevance. VCH, Weinheim.

Minguez, L., Meyer, A., Molloy, D.P., Giambírini, L., 2009. Interactions between parasitism and biological responses in zebra mussels (*Dreissena polymorpha*): importance in ecotoxicological studies. Environ. Res. 109, 843–850.

Mwamburi, J., 2003. Variations in trace elements in bottom sediments of major rivers in Lake Victoria's basin, Kenya. Lake Reserv. Manage. 8, 5–13.

Olsen, P.E., Hogstrand, C., 1987. Subcellular distribution and binding of cadmium to metallothionein in tissues of rainbow trout after exposure to ^{109}Cd in water. Environ. Toxicol. Chem. 6, 867–874.

Olsson, P.-E., Haux, C., 1986. Increased hepatic metallothionein content correlates to cadmium accumulation in environmentally exposed perch (*Perca fluviatilis*). Aquat. Toxicol. 9, 231–242.

Onyari, J.M., Wandiga, S.O., 1989. Distribution of Cr, Pb, Cd, Zn, Fe and Mn in Lake Victoria sediments. Bull. Contam. Toxicol. 42, 807–813.

Oyoo-Okoth, E., Admiraal, W., Osano, O., Hoitinga, L., Kraak, M.H.S., 2010a. Metal specific partitioning in a parasite–host assemblage of the cestode *Ligula intestinalis* and the cyprinid fish *Rastrineobola argentea*. Sci. Total Environ. 408, 1557–1562.

Oyoo-Okoth, E., Admiraal, W., Osano, O., Kraak, M.H.S., Ngure, V., Makwali, J., Orina, P.S., 2010b. Use of the fish endoparasite *Ligula intestinalis* (L., 1758) in an intermediate cyprinid host (*Rastreneobola argentea*) for biomonitoring heavy metal contamination in LakeVictoria, Kenya. Lakes Reserv. Manage. 15, 63–73.

Oyoo-Okoth, E., Admiraal, W., Osano, O., Ngure, V., Kraak, M.H.S., Omutange, E.S., 2010c. Monitoring exposure to heavy metals among children in Lake Victoria, Kenya: environmental and fish matrix. Ecotoxicol. Environ. Saf. 57, 1797–1803.

Poulin, R., 1998. Evolutionary Ecology of Parasites: From Individuals to Community. Chapman and Hall Ltd., London UK.

Raimundo, J., Vale, C., Duarte, R., Moura, I., 2008. Sub-cellular partitioning of Zn, Cu, Cd and Pb in the digestive gland of native *Octopus vulgaris* exposed to different metal concentrations (Portugal). Sci. Total Environ. 390, 410–416.

Sappal, R., Burka, J., Dawson, S., Kamunde, C., 2009. Bioaccumulation and subcellular partitioning of zinc in rainbow trout (*Oncorhynchus mykiss*): cross-talk between waterborne and dietary uptake. Aquat. Toxicol. 91, 281–290.

Sures, B., 2002. Competition for minerals between *Acanthocephalus lucii* and its definitive host perch (*Perca fluviatilis*). Int. J. Parasitol. 32, 1117–1122.

Sures, B., Dezfuli, B., Krug, H., 2003. The intestinal parasite *Pomphorhynchus laevis* (Acanthocephala) interferes with the uptake and accumulation of lead (^{210}Pb) in its fish host chub (*Leuciscus cephalus*). Int. J. Parasitol. 33, 1617–1622.

Tekin-Özan, S., Kir, I., 2005. Comparative study on the accumulation of heavy metals in different organs of tench, *Tinca tinca* (L. 1758) and plerocercoids of its endoparasite, *Ligula intestinalis*. Parasitol. Res. 97, 156–169.

Tenora, F., Barus, V., Kracmars, S., Dvocarek, J., 2000. Concentration of some heavy metals in *Ligula intestinalis* plerecercoides, cestode and *Philometra ovata*, nematodes compared to some of their hosts, Osteichthyes. Helminthology 37, 15–18.

Van Campenhout, K., Infante, H.G., Hoff, P.T., Moens, L., Goemans, G., Belpaire, C., Adams, F., Blust, R., Bervoets, L., 2010. Cytosolic distribution of Cd, Cu and Zn, and metallothionein levels in relation to physiological changes in gibel carp (*Carassius auratus gibelio*) from metal-impacted habitats. Ecotoxicol. Environ. Saf. 73, 296–305.

Wallace, W.G., Luoma, S.N., 2003. Subcellular compartmentalization of Cd and Zn in two bivalves. II. Significance of trophically available metal (TAM). Mar. Ecol. Prog. Ser. 249, 183–197.

Wallace, W.G., Lee, B., Luoma, S.N., 2003. Subcellular compartmentalization of Cd and Zn in two bivalves. I. Significance of metal-sensitive fractions (MSF) and biologically detoxified metal (BDM). Mar. Ecol. Prog. Ser. 249, 183–197.

Wandiga, S.O., 1981. The concentration of zinc, copper, lead, manganese, nickel and fluoride in rivers and lakes of Kenya. Sinet. Ethiopia J. Sci. 3, 1.

Wyatt, R.J., Kennedy, C.R., 1988. The effects of a change in the growth rate of roach, *Rutilus rutilus* (L.), on the population biology of the fish tapeworm *Ligula intestinalis* (L.). J. Fish Biol. 33, 45–57.

Wyatt, R.J., Kennedy, C.R., 1989. Host-constrained epidemiology of the fish tapeworm *Ligula intestinalis* (L.). J. Fish Biol. 35, 215–227.

Xu, Y., Wang, W.-X., 2002. Exposure and potential food chain transfer factor of Cd, Se and Zn in marine fish *Lutjanus argentimaculatus*. Mar. Ecol. Prog. Ser. 238, 173–186.

Zhang, L., Wang, W.-X., 2007. Gastrointestinal uptake of cadmium and zinc by a marine teleost *Acanthopagrus schlegeli*. Aquat. Toxicol. 85, 143–153.