Aquatic Toxicology 124–125 (2012) 34–40

Contents lists available at SciVerse ScienceDirect

Aquatic Toxicology

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

Dynamics of metal uptake and depuration in a parasitized cyprinid fish (*Rastrineobola argentea*)

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ARTICLE INFO

Article history:
Received 17 March 2012
Received in revised form 17 July 2012
Accepted 17 July 2012

Keywords:
Biokinetic model
Cyprinid fish
Metal uptake
Metal depuration
Parasites

ABSTRACT

Infestation of fish by endoparasites may potentially influence metal uptake and elimination by the host. We quantified the metal uptake rate constant (k_u) and efflux rate constants (k_e) of radiolabeled Cd and Co in the cyprinid fish *Rastrineobola argentea* experimentally infected with the parasite *Ligula intestinalis*. During 24 h, the accumulation of Cd and Co increased linearly with no evident steady state in uninfected fish, infected fish and in the parasite. Following aqueous exposures, the k_u for Cd in parasites was about 3× higher than that of infected fish and 6× higher than for the uninfected fish. The k_u for Co was up to 15× higher in the parasites than that of infected fish and 7.5× higher than for the uninfected fish. The k_e for excretion of Cd were consistently higher for the uninfected fish than for the infected fish and also higher for uninfected fish than the parasite. The k_e for Co for the uninfected fish was 1.4–2.0× lower than in the infected fish, but higher for parasites compared to uninfected fish (1.3–2.3×). Pulse-chase feeding experiments with radiolabeled copepods showed that Cd assimilation efficiency from food was higher in infected fish, while Co was assimilated more effectively by uninfected fish. The observed differences in metal dynamics between infected and uninfected *R. argentea* in the laboratory concord with differences in metal concentrations measured in natural populations in Lake Victoria. Our findings provide evidence that *L. intestinalis* infection enhances Cd accumulation, but depletes the essential Co in the cyprinid fish *R. argentea*. We conclude that the combined stress of parasites and pollution changes metal risks to fish hosts in a metal specific manner.

1. Introduction

Fish, like most aquatic organisms, are infected by parasites that have evident detrimental effects on their hosts (Wendelaar Bonga, 1997), caused by deregulation of physiological processes (Barber et al., 2000). These physiological effects of parasites on their hosts may alter the uptake and regulation of metals by the fish. Moreover, parasites themselves may also absorb metals from their host tissues and therefore change the metal accumulation in their hosts (Sures and Siddall, 1999; Sures, 2007) or compete with the host for essential elements (Sures, 2002; Oyoo-Okoth et al., 2010a). A few laboratory and field studies suggested that parasites influence the metal accumulation in their hosts in metal specific patterns (Sures and Siddall, 1999; Oyoo-Okoth et al., 2010a,b; Sures, 2001; Sures et al., 2003).

The cyprinid fish, *Rastrineobola argentea*, exhibit a high degree of infestation with the tapeworm, *Ligula intestinalis* (Cowx et al., 2008). The *L. intestinalis* is a Pseudophyllidean cestode of the family Diphyllobothriidae. In its plerocercoid stage, this tapeworm infests a range of freshwater fish species, particularly members of the Cyprinidae, as its second intermediate host, exhibiting a widespread distribution (Dubinina, 1980; Pierce et al., 2005). Previously, metal partitioning in the cyprinid fish was influenced by the *L. intestinalis* infestation (Oyoo-Okoth et al., 2010a) also at the subcellular level (Oyoo-Okoth et al., 2012a). Whether or not infections with parasites will lead to altered metal uptake and elimination kinetics remains rather speculative though.

Correct estimation of metal uptake and accumulation by aquatic organisms is crucial for an accurate assessment of their potential effects (Veltman et al., 2008, 2010). Such studies can also

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http://dx.doi.org/10.1016/j.aquatox.2012.07.006

provide information for environmental risk of metals in aquatic environments (Wang and Rainbow, 2008). Currently, there is also concern for food safety, which requires knowledge of the accumulated concentrations of metals in fish and their potential risks to humans (e.g. Oyoo-Okoth et al., 2010c). Furthermore, monitoring programs that employ various aquatic species to monitor environmental metal pollution need a better understanding of the processes and significance of metal bioaccumulation in order to interpret the data generated from such programs (Wang and Rainbow, 2008). Therefore, metal accumulation patterns for a wide variety of aquatic organisms have been estimated using radiotracer techniques and quantified by biodynamic models based on a set of first order uptake and elimination rate constants (Pan and Wang, 2009; Croteau and Luoma, 2008; Croteau et al., in press). However, no study has applied radiotracer analysis or used biokinetic models to highlight the metal uptake and elimination in fish infected with parasites. Yet the dynamic interaction between fish, parasites and metals is likely to be important for fish populations in perturbed aquatic ecosystems.

The aim of the present study was to compare the uptake and depuration kinetics of Cd and Co in uninfected and experimentally infected fish. Uptake and depuration experiments were conducted with the cyprinid fish *R. argentea* uninfected and infected with the cestode parasite *L. intestinalis* during short-term laboratory exposure. The uptake and depuration kinetics of ^{109}Cd and ^{57}Co were determined following water-borne and food exposures, combining the radiotracers with different concentrations of Cd and Co.

2. Materials and methods

2.1. Fish sample collection and preparation

A total of about 8000 *R. argentea* (mean weight = 1.50 ± 0.42 g) were collected between April and July 2010 off the coast of Lake Victoria, Kenya (0°12′40″S and 34°49′30″E) and carefully transported to the Kenya Marine and Fisheries Research (KEMFRI) Laboratory Kisumu in Kenya and cultured for 2 months (mean weight of uninfected 18.50 ± 2.42 g and infected 23.50 ± 0.42 g) before the start of the infestation experiments. Fish were reared in race-way type water tanks supplied with filtered lake water (0.45-μm filtered water). The renewal rate was 24 L h^{-1}; salinity: 0.5‰; temperature: 25.2 ± 1.0 °C; pH: 7.4 ± 0.4; dissolved oxygen: >5.0 mg L^{-1}. Water for culturing *R. argentea* was obtained from the least contaminated lake sites.

The first batch of 800 fish were reared in a 1000 L raceway-type tank (designated as tank 1) and another 1000 fish transferred to a second tank (designated as tank 2) for infection with the cestode parasite (*L. intestinalis*). Dissection of 50 fish from each tank, revealed that the natural infection rate of *R. argentea* with *L. intestinalis* was 8.2 ± 3.4% and 7.3 ± 2.1% in tank 1 and tank 2 respectively. During culturing and experimental infection, the fish were fed laboratory cultured zooplankton estimated at 4% of body weight (wet weight per day). Recorded mortality after 4 week of culture was 8%. Before the start of the radioactivity experiments, the mean weight of uninfected and infected fish was 25.50 ± 4.42 g and 31.50 ± 6.42 g respectively.

2.2. Experimental infection

The knowledge of the life cycle of *L. intestinalis* (Dubinina, 1980), was used to achieve infection of *R. argentea* with the parasite. Aquatic birds were trapped with the help of the local fishermen and kept at the KEMFRI laboratory. The infected faeces of the birds (about 85% infection rates) were dropped into tank 2. The infected and non-infested fish were then cultured for 2 months under controlled laboratory conditions until the start of the exposure experiments. Natural infestation of the control group after before the start of the exposure experiments was estimated at 6%. The infection rate in tanks receiving the bird faeces was estimated at 60%. Fish were fed continuously using copepods (*Thermocyclops* spp.) as described below.

2.3. Algae and zooplankton culture

Chlorella spp. culture was maintained for feeding the copepods. Culturing of the algae was performed as described by APHA (1998). The algae were cultured at approximately 10^6 cells mL^{-1}. Measurements of algal density were performed using a hemocytometer (Hausser Scientific, Horsham, PA). Zooplankton was collected from Lake Victoria by horizontal hauls with a plankton net of 200–250 μm mesh size. The plankton samples consisted for about 80% of cyclopoid copepods (*Thermocyclops* spp.). After collection, the zooplanktons were screened through a 500 μm mesh sieve to isolate the size fraction containing dominantly adult copepods. Fish and prawn larvae were removed. The copepods were rinsed for 2 h in a zooplankton washer fitted with 190-μm mesh screen to remove smaller zooplankters such as rotifers, copepod nauplii and non-living components. The mesh size of the zooplankton washer was based on the size of adult cyclopoid copepods. After rinsing, the remaining larger copepods were used to start the laboratory culture. *Thermocyclops* spp. were filtered from the sample and the gravid females were cultured at a rate of about 2000 individuals in 20 L containers (100 copepods L^{-1} of medium), according to standard methods (APHA, 1998). The culture characteristics were 1‰ salinity, 26.5 ± 1.2 °C temperature, 12 h light:12 h dark.

2.4. Determination of metal uptake and depuration

Kinetics of Cd and Co uptake and depuration were studied using radiotracers in the presence of stable metals. The radiotracers were: ^{109}Cd ($t_{1/2}$ = 426.6 d, in 0.1 M HCl) and ^{57}Co ($t_{1/2}$ = 271.8 d, in 0.1 M HCl) from Amersham, UK. The radioisotope additions were 2.5 kBq L^{-1} Cd and 2.0 kBq L^{-1} Co corresponding to a stable metal concentration of 0.04 μg Cd L^{-1} and 0.03 μg Co L^{-1}, respectively. The radioisotopes and stable metal concentrations, control, 0.5, 2.0, 10 and 100 μg L^{-1} Cd or Co, were equilibrated for 12 h before uptake measurements. The uptake experiment was conducted in triplicate. A total of 120 fish per replicate (60 from tanks 1 and 2) were then placed in 25-L aerated medium containing the radiotracers and the stable metals. After 6, 12, 18 and 24 h, 5 fish per treatment were removed from the radioactive media and rinsed twice by transferring from one beaker to another with filtered non-radioactive water for subsequent radiotracer analysis. The infected fish were dissected using stainless steel instruments to remove parasites from the abdominal cavity and the radioactivities of the infected fish and parasites measured. After 24 h of exposure, the remaining fish were placed in non-radioactive, metal free water and allowed to depurate for 21 d. The water was renewed daily. Each 2 d five fish were sampled; the infected fish were dissected and the parasites removed before radioanalysis. The noninfected fish and parasites were analyzed directly.

The assimilation efficiencies (AEs) of metals were measured in a separate experiment in fish feeding on cyclopoid copepods. Seven batches of zooplankton were prepared and maintained during the experiment. The first batch was not contaminated with any metal or radioisotope. Batches 2 and 3 were radiolabeled with 2.5 kBq ^{109}Cd and 2.0 kBq ^{57}Co respectively, without addition of stable metals. In batches 4–7 copepods were incubated with ^{109}Cd and ^{57}Co, respectively, in aqueous solutions of 2, 10 and 100 μg L^{-1} nominal concentration of Cd or Co. All cultures were maintained and handled using trace metal clean techniques to avoid contamination

with metals. The copepods used for feeding ranged 4.50–8.50 mm in length with a dry weight of 3.80 ± 0.2 mg. After 24 h exposure to radiotracers in the aqueous phase, the copepods were collected by a mesh, rinsed with clean water and fed to the fish. A sample was taken for measurements of radioactivity of the copepods. In a pulse-chase experiment, feeding of fish on the radiolabeled copepods lasted for up to 100–120 min. Copepods were added every 20 min to maintain prey density. Immediately following the feeding of *R. argentea* on radiolabeled copepods, fish were placed in non-radioactive water and allowed to assimilate or depurate the ingested food materials for 36 h. Each 6 h, five fish were removed, the infected fish dissected to remove parasites and the fish and parasites were radioanalyzed as before.

2.5. Radioactivity

Radioactivity of fish and parasites were measured using a Wallac 1480 Nai (Ti) gamma counter (Wallac, Turku, Finland). Radioactive disintegrations were counted using a high resolution ϒ-spectrophotometer system composed of three Germanium – N and P type-detectors (EGNC 33^{-1}95-R, Eurysis®) connected to a multi-channel analyzer and computer equipped with spectral analysis software. The detectors were calibrated using appropriate standards for each of the counting geometries used. ϒ-Emissions were detected as follows: ^{57}Co, 122 keV; ^{109}Cd, 88 keV. Counting time was adjusted (typically to around 5 min) to obtain propagated counting errors less than 5% at 1 SD level. Counting usually ranged from 10 to 20 min for radio-analysis of living organisms. Radioactivity of the uptake medium was measured at the beginning and after 6, 12, 18 and 24 h of uptake. For all experiments, the activity in ^{109}Cd and ^{57}Co measured in the whole body of infected and uninfected fish were converted to total Cd and Co with the specific radioactivity (RAS) of Cd and Co in the medium.

2.6. Metal analysis

For the infected fish, metal concentrations were determined after dissection and removal of the parasites. To determine the Cd and Co concentrations in the body, the entire uninfected, infected fish and parasites were dried in a glass tube at 50 °C until constant weight was obtained. This was done for five replicates per treatment. Samples were digested prior to metal analysis as described in Oyoo-Okoth et al. (2010a). For each sample, a maximum of 0.200 g sample was weighed and placed in a Teflon digestion vessel with 7.0 mL of concentrated nitric acid (65%), 1.0 mL concentrated hydrochloric acid (30%) and 1.0 mL hydrogen peroxide (30%). Digests were finally made up with ultra pure de-ionized water to 25.0 mL in acid washed standard flasks. The concentrations of Cd and Co in fish digests were measured with the Thermo electron X7 inductively coupled plasma mass spectrometry (ICP-MS, model X series, UK). The detection limits in (μg g^{-1} dw) were 0.01 for Cd and 0.01 for Co. Reference tissues dogfish muscle DORM-2 (NRCC) were treated and analyzed in the same way as the samples and recoveries ranged from 96 to 98% for Cd and 96 to 101% for Co.

2.7. Data analysis

According to current models, metal concentrations in an organism result from a balance of fluxes over time. Assuming that growth of the organism is negligible during the short-term exposure, water-borne bioaccumulation of metals is described by the first order kinetics (Luoma and Rainbow, 2005). Assuming that the metal elimination is negligible at the beginning of the exposure, uptake (≤24 h) can be expressed as: $I = k_u C_w$, where I is the initial metal flux (μg g^{-1} d^{-1}), k_u is the influx rate constant (L g^{-1} d^{-1}) and C_w is the concentration of metal in the water (μg L^{-1}). When

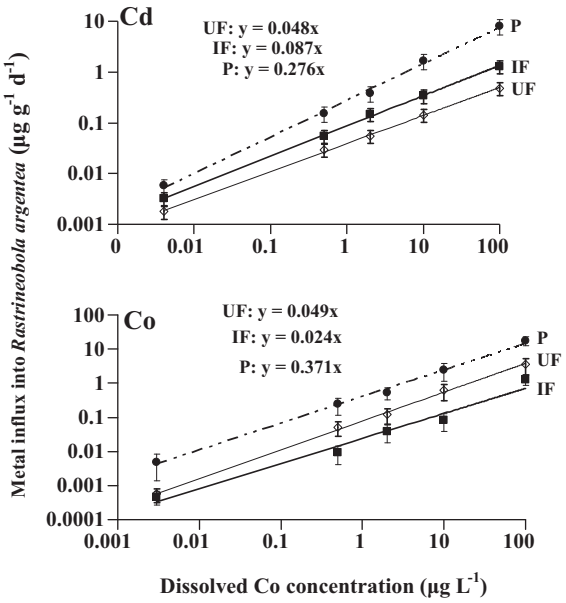

Fig. 1. Cadmium and cobalt concentrations accumulated over 24 h by uninfected fish (UF), infected fish (IF) and parasites (P) from ^{109}Cd or ^{57}Co labeled lake water containing background levels of metal (control) and 0.5, 2, 10, and 100 μg g^{-1} of Cd or Co. Error bars: Standard deviation.

contaminated fish are introduced into the uncontaminated water, metal elimination is defined by: $A_t = A_0 e^{-k_e t}$, where A_t and A_0 are the remaining activities (%) at time t (d) and 0, respectively; k_e is the depuration rate constant (d^{-1}).

3. Results

Before incubation in natural lake water in the aquaria, uninfected *R. argentea* contained 0.38 ± 0.09 μg Cd g^{-1}, while infected fish showed a higher concentration of 0.58 ± 0.11 μg Cd g^{-1}. However, parasites contained the highest concentration of Cd (1.2 ± 0.4 μg Cd g^{-1}) than the uninfected and infected fish. For Co, infected fish showed lower concentrations of 0.21 ± 0.04 μg Co g^{-1} than uninfected fish, containing 0.39 ± 0.04 μg Co g^{-1}, albeit parasites contained the highest concentration of Co (0.64 ± 0.04 μg Co g^{-1}).

During exposure of fish to aqueous metals, influx of cadmium and cobalt into the uninfected, infected *R. argentea* and parasites proceeded linearly with exposure time up to 24 h without any evidence of approaching steady state (Tables 1 and 2). The accumulation of Cd was always significantly ($p < 0.05$) the highest in the parasites than both the infected and uninfected fish. Comparatively, accumulation of Cd was higher in the infected than uninfected fish. Although parasites accumulated Co more than fish, the accumulation of Co was significantly ($p < 0.05$) lower in infected fish than uninfected fish. There was however, an exception for incubations at the highest test concentration of 100 μg Co L^{-1}.

The uptake rates were calculated from radioactivity in fish and concentrations of metals added. The background concentrations of cadmium and cobalt in the water (controls) were respectively 0.04 ± 0.005 μg Cd L^{-1} and 0.03 ± 0.002 μg Co L^{-1}. The relationship between the calculated influx into *R. argentea* and parasites (μg g^{-1} d^{-1}) and the metal concentration (μg L^{-1}) in the water was linear both for the uninfected and the infected fish and for the parasites (Fig. 1). Parasites and fish exposed for 24 h to increasing concentrations of Cd and Co accumulated significant amounts of these metals compared to the controls. The calculated uptake rate constant (k_u) for Cd in parasites (0.276 L g^{-1} d^{-1}) was about three times higher than that of infected fish (0.087 L g^{-1} d^{-1}) and

Table 1
Cadmium concentrations accumulated over 24 h by uninfected fish, infected fish and parasites from ^{109}Cd labeled lake water containing background levels of metal (control) and 0.5, 2, 10, and 100 μg g^{-1} of Cd. Values are means.

Exposure duration (h)	Newly accumulated concentration (ng/L)				
	0 (control)	0.5	2	10	100
Uninfected fish					
0	0	0	0	0	0
6	1.37	5.10	22.20	154.0	1700
12	1.80	9.00	43.40	236.0	2550
18	2.55	14.40	65.20	316.0	3400
24	3.12	19.20	79.10	389.0	4000
Infected fish					
0	0	0	0	0	0
6	0.70	10.60	44.40	220.0	2630
12	0.79	15.80	73.20	348.0	3560
18	1.26	21.70	90.20	532.0	4760
24	1.66	28.80	106.80	820.0	6500
Fish parasite					
0	0	0	0	0	0
6	1.49	10.20	57.70	296	3240
12	2.06	16.60	99.10	487	4485
18	3.69	28.80	127.10	755	5864
24	5.73	48.60	148.70	1202	8208

up to six times higher than for the uninfected fish (0.048 L g^{-1} d^{-1}) ($p < 0.05$). For Co, the infected and uninfected fish also differed in k_u, but opposite to that for Cd. The calculated uptake rate constant (k_u) for Co in parasites (0.371 L g^{-1} d^{-1}) was about 15× higher than that of infected fish (0.024 L g^{-1} d^{-1}) and up to 7.5× higher than for the uninfected fish (0.049 L g^{-1} d^{-1}) ($p < 0.05$). However, the k_u for Co was almost 2× higher for the uninfected fish than for the infected fish.

The depuration of metals from water exposed radiolabeled fish and parasites were measured during 21 d of incubation in clean water (Table 3). Depuration of the metal was similar when fish were exposed to either background concentrations or to 0.5, 2 and 10 μg Cd or Co L^{-1}. The k_e for excretion of Cd were consistently higher (1.5–1.9×) for the uninfected fish than for the infected fish. However, k_e for excretion of Cd was higher for uninfected fish (1.5–2.2×) than the parasite; but exhibited consistently similar ranges between infected fish and parasites at all exposure concentration except the control and at 100 μg L^{-1}. In contrast, the calculated k_e for Co for the uninfected fish was 1.4–2.0× lower than in the infected fish. *Meanwhile* k_e for excretion of Co in parasites were consistently higher than uninfected fish at ambient Co concentrations ranging from 0 to 2 μg L^{-1} (1.3–2.3×). So infected fish tended to retain cadmium and to excrete cobalt to a higher degree than uninfected fish while parasites increased the uptake of Co at lower ambient Co concentrations.

Assimilation of metals via food was measured in a pulse-chase experiment. Depuration of Cd and Co by *R. argentea* and parasites following the 90–120 min pulse feeding on radiolabeled copepods is shown in Table 4. There were no significant differences in metal depuration in fish fed on copepods exposed to different dissolved metal concentrations. The efflux rate constant from food (slope of the regression curve, k_{ef}) for Cd was 2–3× higher for uninfected fish than for infected fish. The k_{ef} of Cd was 1.2–1.8× higher for the parasite than the uninfected fish and was even higher (2.5–5.0×) for the parasite as compared to the infected fish. The k_{ef} for Co was 1.4–2.0× higher in infected than uninfected fish. The k_{ef} of Co ranged from 1.2× to 1.6× higher for the parasite than the infected fish. The observation period of 36 h was assumed to allow for complete digestion of the copepod food, so the radioactivity measured in the fish was no longer present as gut contents. The calculated

Table 2
Cobalt concentrations accumulated over 24 h by uninfected fish, infected fish and fish parasites from ^{57}Co labeled lake water containing background levels of metal (control) and 0.5, 2, 10, and 100 μg g^{-1} of Co. Values are means.

Exposure duration (h)	Newly accumulated concentration (ng/L)				
	0 (control)	0.5	2	10	100
Non-infected fish					
0	0	0	0	0	0
6	0.44	4.21	23.50	75.0	630
12	0.60	8.16	34.60	137.0	1230
18	0.77	11.50	53.50	223.0	1800
24	1.09	16.20	68.80	284.0	2200
Infected fish					
0	0	0	0	0	0
6	0.22	2.40	12.70	30.0	745
12	0.36	5.40	26.90	94.0	1490
18	0.48	7.58	30.00	168.0	2620
24	0.57	9.28	38.80	194.0	3210
Fish parasite					
0	0	0	0	0	0
6	0.78	6.95	37.90	114.1	1082
12	0.99	14.50	59.20	236.6	2094
18	1.34	19.10	98.50	377.9	2997
24	1.98	27.80	133.80	492.8	3689

Table 3
Depuration during 21 d of radiolabeled ^{109}Cd and ^{57}Co in uninfected fish, infected fish and parasite pre-loaded for 24 h with ^{109}Cd or ^{57}Co and 0, 0.5, 2, 10 and 100 μg L^{-1} dissolved metals. Retention: % residual radioactivity in fish (mean ± SEM, n = 40). Values of the exponents correspond to the depuration rate constants.

Exposure concentrations	Cd			Co		
	Percentage retention	Model	R^2	Percentage retention	Model	R^2
Uninfected fish						
0 (control)	21.6	$y = 100e^{-0.073}x$		64.3	$y = 100e^{-0.021}x$	0.992
0.5	21.2	$y = 100e^{-0.074}x$	0.985	57.8	$y = 100e^{-0.026}x$	0.962
2	24.2	$y = 100e^{-0.068}x$	0.955	58.4	$y = 100e^{-0.026}x$	0.973
10	27.8	$y = 100e^{-0.061}x$	0.976	44.6	$y = 100e^{-0.038}x$	0.993
100	42.1	$y = 100e^{-0.042}x$	0.992	20.3	$y = 100e^{-0.076}x$	0.929
Infected fish						
0 (control)	44.6	$y = 100e^{-0.038}x$	0.976	40.4	$y = 100e^{-0.043}x$	0.997
0.5	42.1	$y = 100e^{-0.041}x$	0.943	39.7	$y = 100e^{-0.044}x$	0.984
2	44.7	$y = 100e^{-0.038}x$	0.976	38.7	$y = 100e^{-0.045}x$	0.996
10	46.8	$y = 100e^{-0.036}x$	0.997	32.1	$y = 100e^{-0.054}x$	0.991
100	56.1	$y = 100e^{-0.028}x$	0.965	35.4	$y = 100e^{-0.049}x$	0.903
Fish parasite						
0 (control)	36.7	$y = 100e^{-0.0484}x$	0.991	36.7	$y = 100e^{-0.048}x$	0.965
0.5	44.9	$y = 100e^{-0.038}x$	0.982	44.9	$y = 100e^{-0.038}x$	0.943
2	49.4	$y = 100e^{-0.034}x$	0.9545	47.4	$y = 100e^{-0.036}x$	0.987
10	55.7	$y = 100e^{-0.028}x$	0.944	54.7	$y = 100e^{-0.029}x$	0.965
100	60.3	$y = 100e^{-0.024}x$	0.923	60.3	$y = 100e^{-0.024}x$	0.897

metal assimilation efficiencies (AEs) (% of metal retained after 36 h) were higher for Cd in the infected fish (76–85%) than in the uninfected fish (56–63%) and parasites (40–67%). In contrast, for Co, the AE was lower in infected fish (58–79%) as compared to the uninfected fish (54.2 ± 67%).

4. Discussion

The present study demonstrated that uptake and excretion kinetics of metals in *R. argentea* were modified by infection with *L. intestinalis* and that cadmium and cobalt fluxes showed opposing trends. Using ^{109}Cd and ^{57}Co radiotracers with stable metal addition we quantified the metal uptake rates, efflux rates and the assimilation efficiency in fish and parasites under experimental conditions. The fish originated from Lake Victoria and the experimental infection with the cestode parasites ensured that the two test groups had the same composition and background. The numbers of fish tested were high and the low variability within samples allowed for a robust determination of the parameters k_u, k_e and assimilation % from food in the uninfected and infected fish. Moreover, we removed all cestode parasites so that metals measured in fish could be compared between the infected and uninfected fish.

Metal dynamics in uninfected *R. argentea* showed some differences compared with other fish species, albeit the differences were not very large. Our Cd k_u in uninfected fish was higher than that reported for mangrove snapper *Lutjanus argentimaculatus* (Xu and Wang, 2002), toadfish *Tectractenos glaber* (Alquezar and Markich, 2006), spotted dogfish *Scyliorhinus canicula* (Jefree et al., 2006), and black seabream *Acanthopagrus schlegeli* (Zhang and Wang, 2005). Cadmium taken up by fish is apparently sequestered well; k_e values in *R. argentea* were low in the present study, but were still higher than for *L. argentimaculatus* (Xu and Wang, 2002). The higher accumulation of Cd in infected fish can presumably be accounted for by the modulation of the metabolic activities of the hosts by *L. intestinalis* (Frank et al., 2011), affecting the physiological functioning of the fish causing reduced regulation of Cd uptake. Fish sequesters Cd through metallothionein or other intracellular ligands (Hamilton and Merhle, 1986; Long and Wang, 2005) and lower excretion rates

Table 4
Depuration of ^{109}Cd and ^{57}Co from in uninfected fish, infected fish and parasite following pulse feeding on copepods, pre-loaded with ^{109}Cd or ^{57}Co and 0, 0.5, 2, 10 and 100 μg L^{-1} stable metal. Fish were maintained for 36 h under clean conditions (remaining radioactivity: % after pulse feeding; mean values ± SEM, n = 40). Values of the exponents correspond to the depuration rate constants.

Exposure concentrations	Cd			Co		
	Percentage retention	Model	R^2	Percentage retention	Model	R^2
Uninfected fish						
0 (control)	61.4	$y = 100e^{-0.023}x$		74.3	$y = 100e^{-0.014}x$	0.979
0.5	61.9	$y = 100e^{-0.023}x$	0.985	77.8	$y = 100e^{-0.012}x$	0.992
2	57.4	$y = 100e^{-0.026}x$	0.945	79.2	$y = 100e^{-0.011}x$	0.983
10	56.3	$y = 100e^{-0.027}x$	0.966	74.6	$y = 100e^{-0.014}x$	0.987
100	63.4	$y = 100e^{-0.022}x$	0.972	58.3	$y = 100e^{-0.026}x$	0.953
Infected fish						
0 (control)	83.7	$y = 100e^{-0.008}x$	0.986	65.4	$y = 100e^{-0.020}x$	0.958
0.5	84.8	$y = 100e^{-0.008}x$	0.963	66.7	$y = 100e^{-0.019}x$	0.965
2	82.3	$y = 100e^{-0.009}x$	0.966	62.7	$y = 100e^{-0.022}x$	0.978
10	76.4	$y = 100e^{-0.013}x$	0.989	54.1	$y = 100e^{-0.029}x$	0.974
100	84.9	$y = 100e^{-0.008}x$	0.964	57.4	$y = 100e^{-0.026}x$	0.955
Fish parasite						
0 (control)	40.6	$y = 100e^{-0.043}x$	0.942	76.7	$y = 100e^{-0.013}x$	0.955
0.5	44.8	$y = 100e^{-0.038}x$	0.978	74.9	$y = 100e^{-0.014}x$	0.973
2	45.6	$y = 100e^{-0.038}x$	0.969	67.4	$y = 100e^{-0.019}x$	0.957
10	50.6	$y = 100e^{-0.032}x$	0.944	54.7	$y = 100e^{-0.029}x$	0.975
100	66.9	$y = 100e^{-0.019}x$	0.923	69.3	$y = 100e^{-0.017}x$	0.978

in infected fish might indicate diminished detoxification. Cestodes usually absorb numerous proteins, lipids, ligands and an unknown quantity of other substances from the hosts (Jawale et al., 2011) and presumably reduce the rate of Cd detoxification and excretion from the host. In this study we also show that the cestode increased accumulation of Cd in their body tissues presumably from the external sources and this metal may become bioavailable to the fish for uptake and accumulation.

Cobalt was taken up in uninfected fish at rates lower than cadmium and parasites also accumulated higher concentration of Co at lower ambient Co concentrations. However, the Co assimilation from food was over 60%, showing that the Co that was taken up was retained very well through food pathways. The high assimilation of Co in our pulse-chase experiment accords with observations on carp *Cyprinus carpio* (Van Campenhout et al., 2007), but were higher than observed for zebrafish *Danio rerio* (Liu et al., 2002). Accumulation from food and retention of Co in fish is likely to be based on its role as a co-factor in enzymes and vitamin B_{12} (Da Silva and Williams, 1991). The higher excretion rates of Co in infected fish could be related to the sequestration of Co by the parasites due to competition for this essential metal as we previously observed in field studies (Oyoo-Okoth et al., 2010a).

Several authors, e.g. Luoma and Rainbow (2005) and Croteau and Luoma (2009) proposed a biokinetic model to explore the variability in metal accumulation in situ and argued that experimentally determined uptake and excretion kinetics provide testable predictions. The present observations on the two groups of *R. argentea*, the one infected and the other uninfected by the cestode, and for the parasites, demonstrated that infection enhanced uptake and reduced excretion of cadmium. Yet Co incorporation in fish was shown to be diminished by the cestode and depuration was increased. Based on the experimental results, we expect field populations of infected *R. argentea* to contain high Cd and low Co concentration as compared to uninfected conspecifics. In accordance with this prediction our test populations of infected *R. argentea* collected from the field contained significantly higher body concentrations of Cd and lower concentrations of Co than the uninfected populations. This difference between infected and uninfected *R. argentea* was also observed during independent field observations in 2009 on four subpopulations of *R. argentea* infected to a variable degree with *L. intestinalis* (Oyoo-Okoth et al., 2010a). Thus the predictive value of experimentally determined metal dynamics in fish in absence and in presence of parasites is confirmed by field observations.

The above observation has implications for the fish-parasite host association. Since concerns have been expressed over the high prevalence of *L. intestinalis* in *R. argentea*, the infection of the fish by the parasites may have detrimental effects at the population level of this massively exploited fish species. Cowx et al. (2008) pointed to infection with *L. intestinalis* resulting in late maturation, reduced fecundity and possible increased stress on *R. argentea* by acting on the brain–pituitary–gonadal axis. Also Frank et al. (2011) brought forward that the widespread *L. intestinalis* infections modulate the metabolic activities of their fish hosts. Previously, we demonstrated metal specific patterns in concentration of metals from the fish collected in the field (Oyoo-Okoth et al., 2010a,b). Infection of *R. argentea* with *L. intestinalis* has also been proven to change metal uptake and depuration from both water and food. Although the consequences for the persistence of *R. argentea* populations and its very common parasite *L. intestinalis* cannot be predicted at this stage, increased uptake and low excretion of nonessential metal Cd may have detrimental toxicological consequences for the infected species in highly Cd polluted environment. On the contrary, reduced Co uptake and increased depuration in infected fish may have negative consequences for the uptake and utilization of the essential metal Co especially in environments with low ambient Co concentration. Reports that the availability of metals in Lake Victoria and in fish species has increased in recent years (Mwamburi, 2003; Birungi et al., 2007; Oyoo-Okoth et al., 2010b,c, 2012a,b,c) and the available evidence indicating an interacting inhibitory effects of metals and parasite infection are a common phenomenon, we conclude that these multi-stress conditions change the risk of contamination to the fish host in a metal specific way.

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 laboratory as well as Dr. Philip Kanule of Moi University Atomic Physics Laboratory and Mr. Henk Van Maanen of the International Atomic Energy Agency (IAEA) for their assistance in radioisotope analysis. Departments of Biological Sciences, Wildlife and Fisheries, Moi University provided some of the test animals. Kenya Marine and Fisheries Research Institute (KEMFRI) assisted in the sampling and a laboratory setup.

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