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Energy balance of juvenile *Cyprinus carpio* after a short-term exposure to sublethal water-borne cadmium

L. Ferrari · B. L. Eissa · A. Salibián

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Abstract Stress in fish can be assessed by means of a bioenergetic approach, based on the evaluation of changes in their physiological parameters. The objective of this study was to determine the impact of sublethal water-borne cadmium (Cd) on the energetic balance of juvenile *Cyprinus carpio* under laboratory conditions after a short-term exposure. Fish were exposed to a concentration of Cd (0.15 mg Cd l^{-1}) for 2 weeks. This concentration is environmentally realistic since it is usually found, even at higher values, in heavily polluted periurban water bodies of Argentina. No mortality was recorded among the animals used in the experiments. Food intake, food assimilation and assimilation efficiency, fecal production, liver glycogen content, oxygen consumption, oxygen extraction efficiency, specific metabolic rate, ammonia excretion and ammonia quotient (AQ), condition factor, and liver somatic index were determined. The overall balance was expressed as the scope for growth (SFG). The morphological indices and the liver glycogen content of Cd-exposed fish showed no significant differences when compared to those of controls. There was a significant decrease in the food intake, fecal production, and food assimilation rates as well as in AQ; the SFG exhibited a highly significant decrease. The remaining parameters (assimilation efficiency, oxygen consumption, oxygen extraction efficiency, specific metabolic rate, and ammonia excretion) increased after the exposure to Cd. We concluded that the sub-chronic exposure of *Cyprinus carpio* to a sublethal concentration of Cd causes important alterations in the energy-related homeostasis of fish. Most of the responses are indicative of physiological adaptations to compensate an increased energy requirement due to the impairments caused by the metal.

Keywords *Cyprinus carpio* · Energy metabolism · Scope for growth · Cadmium

L. Ferrari (✉) · B. L. Eissa · A. Salibián
Basic Sciences Department, Applied Ecophysiology Program and Institute of Ecology and Sustainable Development (PRODEA-INEDES), National University of Lujan (UNLu), P.O. Box 221, B6700ZBA Lujan, Argentina
e-mail: lferrari@unlu.edu.ar

L. Ferrari · A. Salibián
Scientific Research Commission (CIC), La Plata, Buenos Aires 1900, Argentina

Introduction

Among divalent metals, cadmium (Cd) is one of the most hazardous biotoxics (Hellawell 1986). When present in the water column, Cd readily accumulates in various tissues, especially in the gills, liver, kidneys, and gonads of fish (Bentley 1991), causing several physiological disturbances (Jezierska and Witeska 2001). The evaluation of the effects of Cd^{2+} on fish is of particular interest since fish are

crucial components of aquatic ecosystems, playing a major role in the food chain. Therefore, it is very important to consider the effect of toxicants on their physiological parameters, to determine the levels of xenobiotics that are compatible with their life, and to detect biomarkers sensitive to the sublethal toxicological effects of pollutant exposure especially at early stages of their development (Sanchez and Porcher 2009).

When comparing results referred to the impact of heavy metals, it appears that the sensitivity of fish as well as the nature of the responses is not uniform among teleosts. This observation leads to wonder about the specificity of the responses of fish when exposed to different heavy metals (De Boeck et al. 1995; Hashemi et al. 2008; Kunwar et al. 2009). Cadmium-exposed fish may show skeletal deformities, alterations in several enzymatic systems, including those involved in neurotransmission, transepithelial transport and intermediate metabolism, alteration of mixed function oxidase activities, abnormal swimming, changes in individual and social behavior, and metabolic disorders, among others (Scott and Sloman 2004; Wright and Welbourn 1994). We have previously shown that exposure of *Cyprinus carpio* to sublethal cadmium concentrations results in gill epithelium damage (Ferrari et al. 2005, 2009), which may lead to alterations in ion and gas exchange and energy balance. At the cellular level, heavy metals can cause a number of adverse effects, such as alterations in the communication between cells and in the interaction with intracellular signal transduction proteins, which may in turn lead to alterations in cell growth and differentiation (Goering et al. 1995). More recently, it has been shown that sublethal Cd also causes important changes in the swimming activity of *C. carpio* in captivity (Eissa 2009; Eissa et al. 2006, 2009, 2010).

In acute water pollution incidents, the physiological disturbances of fish are well known, e.g., respiratory distress, loss of locomotor ability, and behavior alterations. Such responses to environmental stressors have little value as biomarkers because they are insensitive endpoints from the ecosystem perspective and give little information on environmental contamination. In an extreme case, death is indicative that the lethal threshold has been exceeded.

In contrast, when the exposure is chronic or sublethal, biomarkers based on changes in physiological parameters within the natural homeostasis variability associated with biotic and abiotic parameters allow correlating those changes with the effects of exposure to pollutants (Handy and Depledge 1999; Wendelaar Bonga 1997). Biomarkers also serve as indirect indicators of the degree of environmental deterioration and provide a better understanding of how pollutants may affect the overall fitness of the organisms and, eventually, of the structure of the ecological systems (Schelenk et al. 2008; van der Oost et al. 2003). Foreign chemicals may trigger a set of compensatory and adaptive responses in order to restore or readjust the altered processes to their "normal" levels in order to cope with the adverse stressful effects of pollutants (Sibly and Calow 1986).

Among the processes that may be affected by the exposure to heavy metals are the metabolic rate, the excretion of ions (e.g., ammonium), respiration, food consumption, and growth rates (Alves et al. 2006; Hashemi et al. 2008; Wilson et al. 1994).

Gills comprise a large part of the fish body in contact with the external environment and play a key role in gas, ion, and water exchanges between the extracellular fluids and the surroundings (Evans et al. 2005). They also represent an important site of uptake of heavy metals (Ossana et al. 2009), which can be accumulated at levels several orders of magnitude above those found in the environment causing lesions that impair gas and ion exchange (Ferrari et al. 2009; Witeska et al. 2006).

The aim of the present study was to evaluate the effects of semi-chronic exposure to a sublethal concentration of cadmium on a suite of indicators of the energy balance state of juvenile *Cyprinus carpio* under laboratory conditions.

Materials and methods

Test organisms

Juveniles of common carp (*Cyprinus carpio*) of approximately initial wet weight of 5.5–6.0 g, without previous environmental or dietary exposure to pollutants, were obtained from a commercial hatchery. Stock fish were kept in containers with continuously aerated and dechlorinated tap water (TW) from Lujan city (hardness, 80–90 mg $CaCO_3$ l^{-1}) for at least 30 days before use and fed ad libitum "Tetra

Animin" fish food composed of (%): carbohydrates 30.0, proteins 42.7, fat 10.5, ashes 10.5, moisture 6.3, and of a gross energy value of 14.316 J g^{-1}. The analysis of the food showed absence of cadmium. Fish were kept under constant conditions (12 h light/12 h dark photoperiod; 21 ± 2°C) during the holding and experimental periods.

Experimental design

The experiments were carried out in duplicate with a continuous flow-through system, consisting of four 15 l aquaria, two of them with TW (control group) and two with a solution of cadmium in TW (exposed group). Media were delivered to each aquaria from two tanks (one for the control group and other for the exposed group) in a flow-through exposure setup at a rate of 20–25 ml min^{-1} by a Masterflex™ multichannel peristaltic pump. In the corresponding tank, the metal solution was prepared daily by adding $CdCl_2$ (analytical grade, Merck) to the TW to bring the concentration to the nominal level.

The nominal cadmium concentration assayed was 0.15 mg Cd l^{-1}; actual mean concentrations averaged 0.13 mg l^{-1}. The protocol comprised two consecutive periods: acclimation (which lasted 7 days) and exposure (which lasted 15 days). At the beginning of acclimation period, carps from the holding group were randomly divided into groups of five, weighed individually, and placed into each of the four aquaria with TW. The amount of food provided daily in this period was equivalent to 2.5% of the total body mass of fish contained in each aquarium. Food was offered for 2 h and after that the aquaria were cleaned to minimize any fecal ingestion. At the beginning of exposure period, two aquaria continued with TW (control groups), while in the other two TW was replaced by the cadmium solutions (exposed groups). During this period, the daily food ration for all animals was 2% of total wet weight per aquarium (bw). The moisture-free body biomass (dw) was estimated as 27% of bw (Wilson et al. 1994).

The water chemistry during the assay was monitored five times a week in each aquarium; the parameters recorded were temperature, dissolved oxygen (oxymeter Hanna [±0.1 mg l^{-1}]), pH (Mettler pH meter [±0.01]), hardness (Aquamerck test kit, Merck, sensitivity 1 mg l^{-1} $CaCO_3$), and cadmium concentration (mg l^{-1}) using an atomic absorption spectrometer with air/acetylene flame (Instrumentation Laboratory, model 457) at 228 nm.

The following physiological parameters were determined daily in each aquarium:

Food intake (I): food was offered for 2 h; then, the excess was removed by siphoning, filtered, and dried at 60°C to constant weight. Intake was estimated as the difference between the given and the remaining food weight.

Fecal production (F): beginning 24 h after the first feeding, feces were collected by siphoning prior to each food offer, filtered, and dried at 60°C to constant weight.

Assimilation (A) (=Absorption) was calculated as I − F.

Food intake (I), F, and A were expressed as J g dw^{-1} day^{-1}.

Assimilation efficiency (U) (= Utilization) was calculated as U = (I − F/I) * 100 (Alcaraz and Espina 1997) and expressed as percentage.

In order to ensure standard experimental conditions, the last feeding was 36 h before the end of the assay.

At the end of the exposure period, the *oxygen consumption rate* and the *excreted ammonia* in both control and exposed groups were measured individually. For this purpose, each fish was transferred to a plastic vessel containing approximately 300 ml of aerated TW (mean value of 6.25 mg O_2 l^{-1}) with the flow-through switched off. Two samples were taken before and after the containers were sealed for 45 min. The initial and final DO were measured (an additional sample was taken at the end for the determination of the excreted NH_4. The DO was determined by the Winkler method and the excreted NH_4 by a Merck kit (Spectroquant 1.14752); range of 0.03–3.00 mg NH_4 l^{-1}). Oxygen consumption was calculated as the difference between DO_i and DO_f. Oxygen consumption and NH_4 excretion rates were expressed as J g dw^{-1} day^{-1}. Then, each animal was anesthetized in a 0.15 g l^{-1} MS222 (Sigma) solution, weighed, and their length measured by means of a caliper. The liver was dissected, weighed, and its *glycogen content* was immediately determined with the anthrone reagent (Seifter et al. 1950; Sancho et al. 1998). Results were expressed as μg mg $tissue^{-1}$.

The specific metabolic rate (SMR), the oxygen extraction efficiency (OEE), and the ammonia

quotient (AQ) were calculated from the results of the above-mentioned individual measurements as follows: SMR = as mg O_2 g bw^{-1} h^{-1}, OEE (%) = $(DO_i - DO_f)$ * $100/DO_i$ (Espina et al. 2000), and the AQ = the mole to mole ratio of ammonia excreted to oxygen consumed (De Boeck et al. 1995; Owen et al. 1998). The *Scope for Growth* (SFG) was determined as SFG = A − (AQ + SMR) (Roast et al. 1999) and expressed as J g dw^{-1} day^{-1}.

In addition, two non-specific morphological stress indicators, the *condition factor* (CF) and the *liver-somatic index* (LSI), were calculated using the following formulas: CF = ww/$(length)^3$ * 100 and LSI = (liver weight/ww) * 100.

The unit conversions were calculated on the basis of the following equivalences corresponding to ammoniotelic species: 1 Joule = 0.239 cal = 5.94 (cal mg^{-1} $NH4^+$ (NH_4-N), 3.38 cal/mg O_2 (Elliott and Davison, 1975).

Data were presented as mean ± SEM.

Statistical analyses

Assumptions of normality and homoscedasticity were tested with Kolmogorov–Smirnov and Bartlett tests, respectively. The significance of differences between the groups was tested by one-way ANOVA followed by Tukey's test or by the non-parametric Kruskal–Wallis test. For parameters recorded daily in each replicate (I, F, A, and U), a covariance analysis was used to test the association with time (Zar 2010). Data were statistically analyzed with the InfoStat program. The level of significance was set at $P < 0.05$.

Results

During the study, neither unhealthy nor death fish were recorded.

No significant differences were observed in the abiotic parameters of the media, except for the cadmium content (Table 1); DO levels were within the species' tolerance limits. The actual metal concentrations remained constant in the aquaria with Cd, while they were always below the analytical detection limit (0.5 μg l^{-1}) in the control aquaria. The effective cadmium concentration was 86.7% of the nominal concentration.

At the end of the assays, no significant differences were observed in the body weight of both groups of fish relative to the initial values. Similarly, the CF, the LSI, and the liver glycogen content remained unchanged (Table 2).

The values of food intake (I), fecal production (F), and assimilation (A) were significantly lower in the metal-exposed fish; I and A were reduced in a similar magnitude, while F decreased by 50%. In contrast, the assimilation efficiency (U) increased significantly in the Cd-exposed group (Table 3). In all cases, the covariation with the exposure time was not significant.

Cd-exposed fish exhibited a significant increase in the oxygen consumption rate, OEE, and in the SMR, while the scope for growth (SFG) was dramatically decreased. Fish exposed to the metal showed a significant increase in ammonia excretion and a parallel decrease in AQ as compared to controls (Table 4).

Discussion

Cd occurs in periurban contaminated rivers of Argentina over a wide range of concentrations, with values as high as 700–1,700 μg Cd^{+2} l^{-1} (Salibián 2006). Interestingly, these polluted rivers hold a relatively diverse ichthyofauna. So, the concentration of Cd assayed in this work may be considered as environmentally relevant. In our laboratory, under similar experimental conditions, juveniles of *C. carpio* of the same age showed no changes in their survival after a 2-week exposure to 1.0 and 1.6 mg Cd^{+2} l^{-1}(de la Torre et al. 2000; de la Torre 2001). On the other hand, numerous studies have shown that a number of freshwater teleost species of comparable body size were exposed to concentrations close to that used in this work without any evidence of lethality (see Table 5). Considering the range of the metal concentrations assayed, it becomes evident that juvenile carps appear to be resistant to a wide range of Cd concentrations.

Early acute or chronic toxicity studies on the effects of chemicals have adopted survival as the endpoint of the assays. However, other endpoints may be adopted as sensitive and ecologically relevant in sublethal conditions. In the present work, the changes in the whole-body energy balance as biomarkers of

Table 1 Water quality parameters in control and cadmium-exposed groups of juvenile *Cyprinus carpio*

Parameter	Controls		Cd^{2+} exposed	
	Control 1	Control 2	Cadmium 1	Cadmium 2
DO (mg l^{-1})	4.06 ± 0.23 (12)	4.08 ± 0.41 (12)	4.00 ± 0.47 (12)	4.14 ± 0.36 (12)
pH	8.50 ± 0.05 (12)	8.61 ± 0.04 (12)	8.62 ± 0.03 (12)	8.68 ± 0.04 (12)
Hardness (mg $CaCO_3$ l^{-1})	75 ± 2 (5)	74 ± 2 (5)	75 ± 2 (6)	73 ± 2 (6)
Cd^{2+} (mg l^{-1})	ND (11)	ND (9)	0.13 ± 0.03 (11)	0.13 ± 0.03 (9)

Data expressed as mean ± SEM; number of measurements in parenthesis

ND not detected

Table 2 Body weight (BW), body length (BL), morphological parameters (CF; LIS), and liver glycogen content in control and cadmium-exposed juvenile *Cyprinus carpio* measured at the end of the assay

Group	BW (g)	BL (cm)	CF	LSI (%)	Glycogen (μg mg tissue^{-1})
Control	5.76 ± 0 37[a] (10)	7.65 ± 0.14 (10)	1.27 ± 0.03 (10)	0.4 1 ± 0.04 (10)	0.17 ± 0.01 (10)
Cd-exposed	5.94 ± 0. 27[a] (10)	7.66 ± 0.15 (10)	1.42 ± 0.07 (10)	0.47 ± 0.08 (10)	0.19 ± 0.02 (9)

Data expressed as mean ± SEM; number of measurements in parenthesis

[a] Initial body weight (g); 5.49 ± 0.20 (N = 20)

Table 3 Food intake (I), feces production (F), assimilation (A) and assimilation efficiency (U) in control and cadmium-exposed juvenile *Cyprinus carpio*

Parameter	Control group	Cd-exposed group[a]	% Change
Food intake (I) (J g dw^{-1} day^{-1})	1597.59 ± 41.15 (30)	1364.09 ± 84.60* (29)	−14.6
Feces production (F) (J g dw^{-1} day^{-1})	176.36 ± 17.62 (30)	84.60 ± 15.70* (29)	−52.0
Assimilation (A) (J g dw^{-1} day^{-1})	1421.23 ± 41.40 (30)	1280.71 ± 47.26* (29)	−9.9
Assimilation efficiency (U) (%)	88.92 ± 1.07 (30)	93.76 ± 1.34* (29)	+5.4

Data expressed as mean ± SEM; number of measurements in parenthesis

* Indicate statistically significant differences from the control at $P < 0.05$. Last column shows the changes (as %) of Cd-exposed group relative to the mean values of the control group

[a] One sample of feces from the replicate 1 (day 11 of exposure) was lost; thus, the number of determinations for this group was 29

Table 4 Oxygen consumption, oxygen extraction efficiency (OEE), specific metabolic rate (SMR), ammonia excretion (NH_4), ammonia quotient (AQ) and scope for growth (SFG) in controls and Cd-exposed juvenile *Cyprinus carpio*

Parameter	Control group	Cd-exposed group	% Change
Oxygen consumption (J g dw^{-1} day^{-1})	832.39 ± 36.75 (10)	1244.58 ± 52.70* (10)	+49.51
OEE (%)	69.72 ± 4.24 (10)	82.70 ± 2.45* (10)	+18.61
SMR (mg O_2 g bw^{-1} h^{-1})	0.67 ± 0.04 (10)	1.00 ± 0.04* (10)	+49.25
NH_4 (J g dw^{-1} day^{-1})	159.11 ± 8.16 (9)	193.60 ± 9.46* (10)	+21.67
AQ	0.11 ± 0.01 (9)	0.08 ± 0.01* (10)	−27.27
SFG (J g dw^{-1} day^{-1})	459.15 ± 26.62 (10)	-166.05 ± 50.19* (10)	−136.2

Data expressed as mean ± SEM, number of measurements in parenthesis

* Indicate statistically significant differences relative to the control at $P < 0.05$. Last column shows the changes (as %) of Cd-exposed group relative to the means of the control group

Table 5 Cadmium concentration tolerant range of selected teleosts compared to the concentration assayed in this study

Species	Body weight (g)	Assayed concentration (mg Cd^{2+} l^{-1})	References
Carassius auratus	2.5 ± 0.5	10	Battaglini et al. (1993)
Pimephales promelas	1.7 ± 0.3	0.1	Watson and Benson (1987)
Lepomis cyanellus	0.5–5.0	3–11	Carrier and Beitinger (1998)
Mistus vittatus	2.0–3.5	10–50	Datta and Sinha (1990)
Labeo rohita	3.0–5.0	10–50	Datta and Sinha (1990)
Pagrus major	4.69	0.3	Kuroshima et al. (1993)
Cyprinus carpio	3.2 ± 0.4	0.1–10	Abel and Papoutsoglou (1986)
Tilapia aurea	3.1 ± 0.3	0.1–100	Abel and Papoutsoglou (1986)
Cyprinus carpio	0.15	2–9	Suresh et al. (1993)
Cyprinus carpio	5.5–6.0	0.15	This paper

short -term exposure to the metal of a freshwater fish were evaluated.

The degree of toxicity of heavy metals for fish is known to be modulated by biological factors as well as by environmental factors (Fangue et al. 2008; Newman and Clements 2008; Pottinger and Calder 1995). It has also been shown that the stress responses are inherent to the conditions associated with the assays (Strand et al. 2007). In order to reduce the influence of those factors, which may contribute to changes in their relative contribution to the balance of energy sources and thus to misleading results, animals of uniform size were used and the physico-chemical conditions of the media were kept constant throughout the assays (Table 1). The homogeneous experimental conditions of the present study allowed us to conclude that the changes recorded were due to the effect of cadmium. It is important to point out that the only source of Cd for fish was that present in the media. We can thus establish that fish were able to take up the metal mainly through their gills and that Cd bioavailability was constant along time.

The condition factor (CF) and liver somatic index (LSI) are gross non-specific indices indicative of the potential adverse effects of the toxic on the fish health status. CF has been correlated with biochemical, physiological, and reproductive parameters (Eastwood and Couture 2002; Smolders et al. 2002), being an indicator of fish energy reserves. Decreases in CF reflect environmental stress-induced alterations secondary to the presence of chemical pollutants in the environment. Likewise, LSI reflects alterations in the metabolic activity of the liver, acting as an appropriate biomarker of the toxic effect of a polluted environment. In previous studies, juveniles of *C. carpio* exposed to higher concentrations of Cd (0.5 and 2 mg l^{-1}) showed statistically significant differences in both indexes in relation to the controls (de la Torre 2001; Ferrari et al. 2006). The fact that those indexes were not altered after an exposure to a lower sublethal concentration of the metal suggests that neither the fitness of fish nor the metabolism of the liver were affected; the stability of CF and LSI may be attributed to the relatively short time of exposure.

The basis for the use of energetic parameters that accounts for physiological mechanisms to evaluate the impact of environmental stressors is that energy is required to neutralize the effects of toxicants and maintain the animal homeostasis (Giesy and Graney 1989). Internal and external factors may influence the relative value of proteins, lipids, and carbohydrates as energy sources for fish. Since the body weight of both groups of fish did not exhibit significant decreases, we may conclude that the food regime provided sufficient energy for maintenance requirements. It might be concluded that the metal concentration did not cause a stressful condition in terms of energy stores; this was suggested in the particular case of the liver glycogen content of the Cd-exposed fish, which showed that the stress did not trigger its use as a source of energy. This behavior may probably be attributed either to the short period of exposure to the fact that the food provision (with a high content of carbohydrates and proteins) was not interrupted during the assays, or to the fact that *C. carpio* can

store glycogen in liver amounting more than 10% of the tissue weight (Navarro and Gutiérrez 1995). However, it is worth mentioning that Soengas et al. (1996) reported that after an acute exposure of 8 h to a 0.1 mg Cd^{2+} l^{-1} solutions, the liver glycogen of *Salmo salar* was reduced by 72% without changes in the liver weight. In this respect, it is interesting that Asagba et al. (2008) showed that the catfish *Clarias gariepinus* exposed during 7 days to Cd solutions of comparable concentrations with ours did not exhibited accumulation of the metal in the liver.

Food intake behavior in fish was affected by metals under stress conditions (Table 3). In Cd-exposed fish, there was a significant reduction in the food consumption and the fecal production, in spite the fact of the short exposure time to the metal. This reduced food consumption could result in a restriction of the calorie intake with a secondary consequence of an increased energetic cost to partially resist or to compensate the stressor's effect. In addition, the reduction in I was accompanied with a reduction in A in a comparable magnitude possibly because of the damage in the intestinal epithelium (Peters 1982), which was compensated only partially by a modest increase in the dietary assimilation efficiency (U). In contrast, the percentages of reduction observed suggest that the effect on the fecal production of Cd-exposed fish was not proportional to the reduction in the food consumption. The reduction in the appetite appears to be an effect attributable to the metal, which may be related to the possibility of a decrease in the sensitivity of the olfactory nerves to the nutrients of the food (Sloman and Wilson 2006). The data presented in Table 3 suggest that the reduction in fecal production may be dissociated from that of the assimilation and that other toxic effects must be responsible, either directly or indirectly, for such effect, probably tending to compensate the energetic deficit secondary to a reduction in food consumption. This compensation seems to have been effective, as it appears from the fact that body weight of fish was not altered at the end of the exposure to the toxic. In addition, the lack of covariation between I, F, A, or U and time may indicate that changes were cadmium-induced responses.

It is interesting to point out that the animals exhibited those significant changes, before a modification in other indicators such as CF, indicating adverse effects in the general condition of animals. This suggests that these changes would be secondary to the metabolic adjustment caused by the metal, as part of the homeostatic response of the animal to the stress.

Moza et al. (1995) reported that *Carassius auratus* exposed to 0.05, 0.15, and 0.30 mg Cd l^{-1} showed decreased I, U, and specific growth ratio (SGR). These authors assumed that as the exposure time to Cd is lengthened the feed conversion efficiency, associated with increasing metal levels, may provoke a poor utilization of food reflected in a negative effect on the SGR. Fish fed diets with As, Cd, Cu, Pb, and Zn also exhibited decreased food intake, growth, and survival rates. De Boeck et al. (2000) showed that the exposure of *C. carpio* to a salt stress provoked a reduction in the food consumption.

In the present study, carps showed an important increase in the standard metabolic rate, which was evidenced by a parallel increase in oxygen consumption and a subsequent increase in oxygen extraction efficiency (Table 4). These results suggest a compensatory response to an early increased demand for energy. It is interesting that these responses of critical physiological parameters to the stressor appeared early, after a relatively short period of exposure.

Some fish respond to metals by regulating their standard metabolic rate as an adaptive reaction. Couture and Kumar (2003) observed a decrease of 25% in the metabolic rate of *Perca flavescens* exposed to cadmium-contaminated water from Canadian lakes. The exposure of juvenile *Oncorrhynchus mykiss* to sublethal concentrations of aluminum caused a decrease in food intake, but not in the metabolic rate (Allin and Wilson 1999). In contrast, *Pimephales promelas* exposed to 1, 1.5, and 2.0 mg Cd^{2+} l^{-1} decreased its metabolic rate by 30–60% (Pistole et al. 2008). *Oreochromis niloticus* exposed to 0.35, 0.75, 1.5, and 3.0 mg Cd^{2+} l^{-1} exhibited metabolic alterations, decreased glycogen content, and glucose uptake in white muscle (Almeida et al. 2001). Similar results were obtained after exposure of *C. carpio* to sublethal concentrations of copper (De Boeck et al. 2006).

Proteins play a central role in the energy production during the stress caused by toxicants. In this study, the results indicate that although the aerobic energy metabolism increased, growth was not affected, suggesting a possible reallocation of energy use from growth to other processes in order to readjust

metabolism to cope with other effects of Cd (Wendelaar Bonga 1997), maintaining the basal titers of biotransformation enzymes as well as their induction (Morrow et al. 2004). The particular contribution of protein catabolism to the total energy production of freshwater fish can be assessed by the determination of the ammonia quotient (AQ). Most of the end products nitrogenous metabolism result from protein catabolism, ammonia being the principal one. Thus, AQ estimates the proportion of proteins involved in respiration evaluated as the oxygen consumption rate (De Boeck et al. 1995; Owen et al. 1998). The decrease in AQ observed in the cadmium-exposed group (Table 4) may indicate that the increase in the energy requirements was possibly compensated by a partial stimulation of the protein catabolism, being carbohydrates and/or lipids the alternative aerobic sources used to overcome the stress due to the metal exposure. This response could be incipient, as suggested by the increases in oxygen consumption and OEE recorded. In other words, the rate of protein breakdown may not be acute because of those compensatory responses, together with the fact that the oxygen concentration in the water remained constant, thus excluding the possibility of a switch to an anaerobic degradation of proteins. Besides, the AQ in fish that degrade proteins aerobically is much higher than that measured by us; the quotient may be reduced in its magnitude when other nutrients are used (Kutty 1978). Juveniles of *C. carpio* exposed to copper behave in a similar way (De Boeck et al. 1995), while adult females of the same species exposed to either acute or chronic concentrations of cadmium showed an increase in protease activity, free amino acids, and aminotransferase activity in the liver and kidneys (De Smet and Blust 2001). Assays performed under experimental conditions almost identical to ours, *C. carpio* exposed for 14 days to a much higher concentration of the metal showed a 60–98% increase in liver transaminases activities, thus indicating that cadmium might induced the activation of the amino acid catabolism (de la Torre et al. 1999).

The growth of living systems depends on the net balance between the dietary energy intake, the energy used for metabolic and stress-related processes, the energy lost in the excreta and feces, and on the interplay of different physiological processes (digestion, assimilation, respiration, and excretion). Thus, growth can be affected by interfering substances with the overall stability and functioning of these processes. On this basis, the effects of pollutants on the energy balance and animal growth could be analyzed using an integrated approach and quantified by means of the scope of growth (SFG). This parameter has the advantage of integrating the responses to a range of biomarkers into a single value that reflects the energy status of the animal (Widdows and Salkeld 1993), being an indirect non-specific indicator of the levels of pollution in the environment in relation to the maintenance of the energy balance, providing information on its impact on the ability to survive, grow, and reproduce (Widdows and Donkin 1991). A decrease to negative values in SFG due to exposure to different pollutants has been previously reported in fish (Alcaraz and Espina 1997; Espina et al. 1986; Strand et al. 2007) as a clear indication of an acute reduction in the energy available for growth using the energy reserves. Changes in the SFG of fish are indicators of great ecological importance that has proven a sensitive index of stress during exposure to metals. In the present study, this parameter decreased significantly after a 2-week exposure to sublethal concentrations of cadmium. This result suggests an energy deficit that would account for the increases in oxygen-related parameters (SMR, OEE) and in the AQ.

The analysis of our results shows that the main effects of the exposure of *Cyprinus carpio* to 0.15 mg Cd/l^{-1} on the energy metabolism were compensatory. The responses observed can be related to the *general adaptation syndrome* (Seyle 1973), which states that a number of quantifiable responses under stress conditions contribute to resisting the adverse effects of a toxicant for a finite period of time.

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