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Cadmium effects on some energy metabolism variables in *Cnesterodon decemmaculatus* adults

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Abstract This work is focused on the responses of some energy metabolism variables in *Cnesterodon decemmaculatus* adults exposed to cadmium under controlled laboratory conditions. This species has been used as bioindicator for evaluating the effects of different chemicals on diverse biological processes and is frequently used as test organism in ecotoxicity studies that include cadmium as reference toxicant. Animals were exposed for 12 days to the following concentrations: 0, 0.45, and 0.8 mg Cd/L. Food intake, fecal production, specific assimilation, condition factor, mortality percentage, oxygen consumption, oxygen extraction efficiency, specific metabolic rate, ammonia excretion, and ammonia quotient were measured. The overall balance was expressed as scope for growth (SFG). Cadmium-exposed groups showed a significant decrease in food assimilation and condition factor at the end of the exposure. There was an increase in specific metabolic rate and a decrease in SFG in the group exposed to 0.8 mg Cd/L. The condition factor and the SFG appeared as sensitive biomarkers of health status and growth of the animals, respectively. Cadmium-exposed fish reduced food intake, which was reflected in a decreased assimilation with concomitant decline in the external energy supply from feeding. Our results highlight the importance of considering the metabolic status of the test organisms when analyzing the responses of the biomarkers usually used as effect parameters in ecotoxicological evaluations under experimental conditions.

Keywords *C. decemmaculatus* · Energy metabolism · Cadmium · Food intake · Condition factor · Scope for growth

Introduction

Chemical contamination has become a major concern worldwide because it affects all the components of the biosphere, leading to a diminished quality of life. The U.S. Environmental Protection Agency (USEPA 2001) included 13 of the 59 metal elements in the National Priorities List, headed by lead (CASRN 7439-92-1), arsenic (CASRN 7440-43-9) and cadmium (CASRN 7440-43-9). These metals are extremely hazardous to human health, have no biological functions, and tend to accumulate in living organisms as they are not eliminated by biotransformation mechanisms. Although cadmium is a non-essential element, it follows the metabolic pathway of essential metals such as zinc, copper, and calcium (Mebane 2006). It is used as reference toxicant in ecotoxicological assessment studies, and its toxic effects have been often evaluated in the test species such as the one used in this study (de la Torre et al. 1997; Mastrángelo and Ferrari 2013).

The cadmium (Cd) market started in the mid-20th century with the manufacturing of rechargeable nickel

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cadmium batteries; currently it is used in galvanizing, photographic emulsions, printing inks and as color pigment in paints, among others. Since 1990, global consumption has remained constant, at about 20,000 tons per year (UNEP 2010). Cadmium has been classified as a human carcinogen (group 1) by the International Agency for Research on Cancer of the World Health Organization (WHO 1992; IARC 1993; Arroyo et al. 2012), exerting its cytotoxic effects through apoptosis and necrosis (Rani et al. 2014). Moreover, cadmium-exposed fish may show skeletal deformities, alterations in several enzymatic systems, metabolic disorders, among others and changes in social and swimming behavior (Mebane 2006; Pereira et al. 2016; Abdel-Tawwab and Wafeek 2017). Although the levels of Cd in surface river water do not exceed guideline values (<0.25 µg/L, SRHN 2003), values of cadmium as high as 0.7–1.7 mg/L have been reported in surface water in Reconquista river of Argentine (Salibián 2006), alerting about its potential effects in the freshwater biota.

All living organisms require energy for growth, reproduction and maintenance of metabolic homeostasis. Energy supply is a limiting factor for physiological processes. Under stress, energy demand increases, and growth and reproduction may be compromised if the animal cannot compensate for dietary imbalances (Rueda-Jasso 2004). Studies on energy balance in aquatic animals are useful to elucidate how energy is invested in homeostatic maintenance, growth and gonadal production, and how these vary in response to environmental conditions. An individual's growth is determined by the balance between energy and biomass, and is affected by the energy source and the quality and quantity of nutrients in the diet (Rosas et al. 2003). Physiological processes in teleost respond to environmental factors such as water quality, food availability, temperature, dissolved oxygen, and their combination (Liew et al. 2012).

Bioenergetic parameters estimates the energy required to neutralize the effects of toxicants and maintain the animal homeostasis. Exposure to heavy metals may affect the metabolic rate, the excretion of ions (e.g., ammonium), respiration, food consumption, and growth rates (Alves et al. 2006; Hashemi et al. 2008). In fish, the specific metabolic rate (SMR) is an important physiological parameter because it integrates quantitatively the energy interconversions that take place in anabolic and catabolic processes (Ferrari et al. 2011).

Exposure to single toxic agents or in combination may have a negative impact on feeding behavior either directly or by causing damage to the sensory, nervous and/or endocrine systems, leading to an imbalance between energy supply, and demand. Feeding is ultimately the result of a balance between hunger, appetite, and satiety. Hunger is the physiological need for food and constitutes a strong motivation to feeding behavior, which consists of searching for and/or ingesting food. Appetite is the desire to eat food, which is usually associated with sensory (sight, smell, taste) perception of food. In fish, feeding behavior is often an indicator of appetite as it is associated with an increase in locomotor/swimming activity and in searching for food. The effect of toxicants on any of the above variables will undoubtedly reduce food intake. Moreover, the presence of xenobiotics in the environment causes alterations in the endocrine control of feeding (Hoskins and Volkoff 2012).

The ten spotted livebearer fish, *Cnesterodon decemmaculatus* (Jenyns, 1842, Pisces, Poeciliidae, Cyprinodontiformes) is a Neotropical fish widely distributed in Argentina, Chile, Paraguay, Brazil, and Uruguay. It reaches high densities, often dominates the fish assemblages inhabiting shallow-water environments of southern South America (Gómez et al. 1998) and occurs in habitats that vary from pristine to degraded (Hued and Bistoni 2007). This species is small-bodied, omnivorous, livebearer, has a marked sexual dimorphism and a very efficient reproductive strategy. In addition, it has a short life cycle and fast growth. These traits, together with the fact that it can be easily bred in the laboratory make *C. decemmaculatus* a suitable test organism for ecotoxicity bioassays at the regional level. It proved to be a reliable bioindicator for evaluating the effects of different chemicals on diverse biological processes (de la Torre et al. 2005; Menéndez-Helman et al. 2012; Vera-Candioti et al. 2014). *C. decemmaculatus* was recommended for ecotoxicity tests (IRAM 29112/2008), and a standardized laboratory rearing procedure was developed for experimental purposes (Somma et al. 2011). For *C. decemmaculatus* ecotoxicological studies, cadmium was proposed as reference toxicant, both for lethal and sub lethal response (Mastrángelo and Ferrari 2013; Ossana et al. 2016), being important to increase the knowledge about the species responses to the metal exposure.

The aim of the present study was to evaluate the effects of exposure to cadmium on a suite of energy balance indicators in adults of *Cnesterodon decemmaculatus* under laboratory conditions and evaluated the potentiality of the metabolic parameters as indicator of environmental stress.

Materials and methods

Test organisms

We used stock fish, mostly adult females, of *Cnesterodon decemmaculatus* with a mean weight of 125.49 ± 13.36 mg and a standard length of 27.42 ± 0.50 mm (total $N = 141$; mean ± SEM). They were reared in aquaria filled with continuously aerated, dechlorinated tap water (pH 8.07–8.54; hardness: 60–95 mg $CaCO_3$/L; alkalinity: 380–450 mg

$CaCO_3$/L). The culture temperature (23 ± 1 °C) and photoperiod (16:8 L:D) were similar as those used for assays with the test specie according to IRAM 29112 (2008). During reared there was maintained approximately swimming surface of 16 cm^2/adult fish (Somma et al. 2011) and daily *ad libitum* feeding. Same controlled temperature and photoperiod conditions were maintained during the experiment.

Experimental design

Two separate experiments of Cd exposure were done with the same experimental design: A1, in spring and A2, in winter. The exposure media was reconstituted, moderately hard water (MHW; pH 7.4–7.8; hardness: 80–100 mg $CaCO_3$/L; alkalinity: 60–70 mg $CaCO_3$/L; USEPA 1993) was used for the dilutions and control media.

Before the experiments began, fish were subjected to an acclimation period of 14 days: animals were randomly distributed into 15 × 15 × 20 cm aquaria containing 2 L of MHW, 10–15 fish each one, with a mean loading of approximately 0.5 g fish/L. Aquaria were placed in a chamber with controlled temperature and photoperiod (23 ± 1 °C, 16:8 L:D) and continuous aeration. Fish were provided food *ad libitum*.

In A1 and A2 fish were exposed for 12 days at mean effective concentrations of 0.45 mg Cd/L MHW and 0.8 mg Cd/L MHW, respectively. The cadmium solutions were prepared from a stock solution of $CdCl_2$•2.5 H_2O. The concentration range was chosen according mortality register in previous screening assays. Each assay was performed with a parallel control group in MHW. The assays were performed in triplicate (ten animals/replicate) with medium renewal every 96 h to avoid falling exposure concentration. Cadmium concentrations were measured daily in each replicate of exposed groups and checked sporadically in control media. The percentage of mortality was recorded for each case.

At the beginning of the exposure period fish were randomly assigned at control or Cd-exposed group. In order to reduce stress by handling the total number of fish assigned to each replicate was weighed together to obtain the total biomass/replicate. For each replicate, the amount of food provided daily was equivalent to 2% of the total body mass of fish contained in each aquarium. The correction of the biomass by mortality was performed daily.

Fish food (TetraFin goldfish flakes) was slightly ground and dried to constant weight (60 °C) for subsequent weighing and fractionation. Fish food composition was: protein 41%, fat 14.8%, carbohydrate 27.1%, ash 8.1%, and moisture 9 mg/g, with energy content of 1.823 J/mg.

Except for the amount of food offered, the experimental conditions remained constant from the beginning of the acclimation period to the end of the study.

Measured parameters

For each replicate, dissolved oxygen (oxymeter Hatch), pH (pH meter Mettler), hardness (Aquamerck test kit, sensitivity 1 mg/L $CaCO_3$) were measured and water samples were taken to determine Cd concentrations. The effective concentration of Cd in the solutions assayed was measured by atomic absorption spectrophotometer (Perkin Elmer; AAnalyst 200 model, quantification limit: 0.048 ± 0.009 ppm) equipped with hollow cathode lamps (λ = 228.8 nm).

The following biological parameters were recorded daily in each aquarium:

Mortality, expressed as percentage of cumulative mortality over time per treatment.

Estimated food intake (I): prior to each food offer, dead fish were removed from the aquarium and weighed to adjust feed ration according to updated biomass. Food was offered every morning for 1 h and any remaining debris was removed by siphoning, filtered, and dried to constant weight at 60 °C. Food intake, expressed as mg food/mg biomass/day, was estimated as the difference of weight between the offered and the remnant amount of food.

Estimated fecal production (F): feces were collected by siphoning prior to each food offer, filtered, and dried to constant weight at 60 °C. It was expressed as mg feces/mg biomass/day.

Estimated specific assimilation (A) was calculated as A = I−F (Alcaraz and Espina 1997).

All parameters were transformed to energy units and expressed as mJ or J/mg biomass/day.

At the end of exposure, and after 24-h starvation period, a sub-sample of individuals of control and other sub-sample of exposed group, randomly selected, were assigned to the determination of condition factor, oxygen consumption, and ammonia excretion. For the *Fulton's* condition factor (*K*), the body weight (mg) and standard length (mm) were measured and *K* was calculated as: (wet weight/length^3) * 100. Some of these animals were previously used for the respirometer measurement.

The oxygen consumption and ammonia excretion were measured as follows: each specimen was individually introduced into a hermetically closed acrylic respirometer supplied with aerated MHW in an open flow system for 1 h (acclimation period). Then, the flow in the respirometer system was closed for 1 h and dissolved oxygen (DO) was measured at initial (i) and final (f) times using a Hach oxygen sensor (detection limit 0.1 mg/L). Additional samples were taken for determination of ammonia concentration at initial and final times when fish were in the closed-system respirometer. Finally, animals from each respirometer were individually weighted. Oxygen consumption was calculated as the difference between DO_i and DO_f in each respirometer. Water samples for ammonia determination were

stored at −20°C for 72–96 h until analysis. Ammonia was measured with an ion-specific sensor (Mettler Toledo, sensitivity ± 2%) and excretion was expressed as µg NH_4^+ per g wet weight/h.

The specific metabolic rate (SMR), was calculated as mg O_2 consumed/g wet weight/hour, and oxygen extraction efficiency as OEE (%) = (DO_i − DO_f) * 100/DO_i (Espina et al. 2000).

The ammonia quotient (AQ) was calculated as mole to mole ratio of ammonia excreted to oxygen consumed (De Boeck et al. 1995; Owen et al. 1998).

The estimated scope for growth (SFG) was determined as = A−(R + N), where A is specific assimilation, R is O_2 consumption and N is ammonia excretion (Verslycke et al. 2004), and expressed as Joule/g wet weight/day.

Statistical analysis

For each assay, the evaluation of the effect of Cd on the parameters considered was compared to parallel control in each assay. Accumulated mortality and the estimated food intake (I), fecal production (F) and specific assimilation were evaluated at day 6 and day 12 of exposure. Assumptions of normality and homoscedasticity were tested with the Kolmogorov–Smirnov test and the Bartlett test, respectively. The significance of differences between the groups was tested using a one-way ANOVA followed by Tukey's test or the non-parametric Kruskal–Wallis test (Zar 2010). Data were statistically analyzed with the InfoStat program (Di Rienzo et al. 2014). The level of significance was set at $P < 0.05$.

Results

The levels of dissolved oxygen and pH were suitable, resulting in no additional stress for any treatment (Table 1). Except for the cadmium content during the exposure phase, all physico-chemical parameters and replicated remained constant throughout the study. This developed comparable results between treatments and replicates.

In each assay, the used animals were homogeneous with a mean body weight and SD at initial time of exposition, of 93.02 ± 30.14 mg ($N = 35$) and 122.29 ± 19.96 ($N = 39$) for A1 and A2, respectively, measured in a pool of animals. In A2, at day four of the exposure period one of the replicates of the Cd group was missing, so we process this treatment in duplicate. After a 6-day exposure in Cd group mortality was 20 % for A1 (0.45 mg Cd/L) and 25% for A2 (0.8 mg Cd/L). At day 12 of exposure mortality increased to almost 50% for both assays, A1 and A2. Although mortality of the A1 control group was slightly higher than expected, it was significantly lower than the exposed group (Table 2). The Cd-exposed groups in the two assays showed a significant decrease in K compared to their parallel control.

Compared to its controls, at day 6 of exposure there is no effect in I, F and A ($P < 0.05$), while at day 12 of exposure A1 (0.45 mg Cd/L) and A2 (0.8 mg Cd/L) shown a significant reduction for the three feeding-related parameters (Table 3). Although there is no clear trend of time effect on the indicated parameters, the results suggest a delayed effect over time.

The values of SMR, OEE %, ammonia excretion (NH4 excretion), AQ and SFG calculated for A1 and A2 at day 12 of exposure are shown in Table 4. Considering that the animals from both assays were adults and came from the same laboratory culture, the differences between the controls may be attributes to seasonal variations, since A1 and A2 were conducted in spring and winter, respectively.

No significant differences in any of the parameters were found between the exposed and control groups for A1, while A2 showed a significant increase in SMR and a significant decrease in SFG.

Table 1 Water quality parameters in the control and Cd-exposed groups of *C. decemmaculatus*

Parameter	A1		A2	
	Control	0.45 mg Cd/L	Control	0.8 mg Cd/L
pH	7.43 ± 0.19	7.38 ± 0.12	8.13 ± 0.41	8.05 ± 0.34
	(6)	(10)	(11)	(6)
Disolved oxygen (mg/L)	7.66 ± 0.25	7.49 ± 0.39	8.03 ± 0.52	8.11 ± 0.79
	(6)	(10)	(11)	(6)
Hardness (mg $CaCO_3$/L)	94.17 ± 4.91	97.0 ± 4.22	87.06 ± 8.72	87.06 ± 8.72
	(6)	(10)	(16)	(16)
Cd (mg/L)	ND	0.445 ± 0.043	ND	0.778 ± 0.094
	(5)	(36)	(6)	(24)

Data expressed as mean ± SEM; quantity of water samples measured in parenthesis

ND not detected

Table 2 *C. decemmaculatus* adults exposed to Cd and parallel control groups (MHW): cumulative mean mortality at 6 and 12 days exposure and mean values of body weight, standard length and condition factor (K) recorded at day 12 of exposure

Parameters	A1		A2	
	Control	0.45 mg Cd/L	Control	0.8 mg Cd/L
Mortality (%)	8.89 ± 3.95	19.07 ± 3.76*	0	25.82 ± 2.75*
6 days	(3)	(3)	(3)	(2)
Mortality (%)	22.03 ± 4.61	46.02 ± 10.74*	7.72 ± 7.31	51.65 ± 8.95*
12 days	(3)	(3)	(3)	(2)
Body weight	131.6 ± 9.1	110.16 ± 15.13	140.61 ± 7.74	119.6 ± 4
(mg)	(9)	(8)	(14)	(10)
Standard length	27.26 ± 1.02	26.82 ± 1.26	27.63 ± 0.49	27.98 ± 0.54
(mm)	(9)	(8)	(14)	(10)
K	0.66 ± 0.04	0.55 ± 0.03*	0.67 ± 0.03	0.55 ± 0.02*
(ww/length^3) * 100	(9)	(8)	(14)	(10)

Data expressed as mean ± SEM; in parenthesis number of replicates for mortality or number of animals (for the other parameters)

* Denotes significant differences with respect to the parallel control group of each assay ($P < 0.05$)

Table 3 Estimated food intake (I), estimated fecal production (F) and estimated specific assimilation (A) (J/g ww/d) calculated for adults of *C. decemmaculatus* exposed to Cd and control groups (MHW) at days 6 and 12 of exposure

Parameters	A1		A2	
	Control	0.45 mg Cd/L	Control	0.8 mg Cd/L
I (6 days)	26.82 ± 0.97	22.5 ± 1.19	24.08 ± 1.27	21.19 ± 1.68
	(18)	(18)	(18)	(12)
F (6 days)	7.62 ± 0.56	4.56 ± 0.69	5.03 ± 0.8	2.31 ± 0.98
	(18)	(18)	(18)	(12)
A (6 days)	19.02 ± 1.02	17.94 ± 1.25	19.77 ± 1.44	18.89 ± 1.76
	(18)	(18)	(18)	(12)
I (12 days)	29.07 ± 0.86	20.09 ± 1.21*	25.03 ± 0.73	18.66 ± 1.01*
	(31)	(33)	(30)	(22)
F (12 days)	6.54 ± 0.58	3.67 ± 0.52*	3.78 ± 0.58	0.98 ± 0.69*
	(31)	(24)	(30)	(22)
A (12 days)	22.52 ± 0.89	17.23 ± 1.31*	21.78 ± 1.06	16.03 ± 1.29*
	(31)	(27)	(28)	(22)

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

* Denotes significant differences with respect to the parallel control group ($P < 0.05$)

Discussion

An understanding of aquatic animal's responses to pollutants is important for the development of strategies to prevent and detect environmental risk hazards, for instance cadmium, a nonessential metal for aquatic organisms. Cadmium-exposed vertebrates have been reported to show changes in swimming activity, and behavior (Sloman et al. 2003), hypocalcemia, alterations in gill morphology (Ferrari et al. 2009), increased antioxidant enzyme activity (De Almeida et al. 2003; Ossana et al. 2009) and increased micronuclei frequency (Alves et al. 2006; Ossana et al. 2009) among others. Our work is focused on the responses of some energy metabolic variables in *C. decemmaculatus* adults exposed to different concentrations of cadmium under controlled laboratory conditions.

Considering that effects of metals are modulated by biological and environmental factors and stress responses depend on the experimental conditions (Strand et al. 2007; Ferrari et al. 2011), our assays were conducted under similar experimental conditions, using comparable test animals from the same laboratory-reared stock. Moreover, cadmium concentrations in the exposure solutions remained constant throughout the exposure period. These considerations allow us to assume that the differences between the exposed and controls can be attributed to the effects of Cd.

Several studies dealing with adult and juvenile *C. decemmaculatus* and other teleost species acutely and chronically exposed to sublethal cadmium concentrations (de la Torre et al. 2007; Ferrari et al. 2011; Morales Cazan and Klerks 2015; Vergilio et al. 2014; Peles et al. 2012) have demonstrated adverse enzymatic and physiological effects on different biomarkers. The fact that *C. decemmaculatus* is frequently used as test organism in ecotoxicity studies at the regional level and that cadmium is selected as a reference toxicant emphasizes the value of characterizing early effect endpoints.

The cadmium-exposed fish showed a significant decrease in the feeding-related parameters (A, I, and F) at final

Table 4 Specific metabolic rate (SMR), oxygen extraction efficiency (OEE %), ammonia excretion, ammonia quotient (AQ) and scope for growth (SFG) calculated for adults of *C. decemmaculatus* exposed to Cd and control groups (MHW) at day 12 of exposure

Parameters	A1		A2	
	Control	0.45 mg Cd/L	Control	0.8 mg Cd/L
SMR (mg O_2/g ww/h)	0.49 ± 0.08 (8)	0.51 ± 0.14 (8)	0.26 ± 0.03 (10)	0.59 ± 0.08* (8)
OEE (%)	3.44 ± 0.72 (8)	3.72 ± 0.94 (8)	9.93 ± 1.11 (10)	10.73 ± 0.96 (8)
Ammonia excretion (μg NH_4^+/g ww/h)	39.7 ± 5.31 (7)	25.14 ± 3.98 (7)	23.83 ± 4.75 (6)	39.28 ± 15.3 (4)
AQ	0.18 ± 0.02 (7)	0.15 ± 0.04 (7)	0.19 ± 0.03 (6)	0.14 ± 0.05 (4)
SFG (J/g ww/d)	54.46 ± 8.57 (9)	41.84 ± 8.46 (6)	112.17 ± 27.84 (6)	−77.94 ± 42.28* (4)

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

* Denotes significant differences with respect to the parallel control group ($P < 0.05$)

exposure time, together with a mortality of about 50%, and a significant decrease in the Fulton's condition factor, K (Tables 2, 3). These results suggest that the cadmium exposed animals may affected their metabolic homeostasis over time. The lower assimilation values obtained for the animals exposed to cadmium may have resulted from an indirect effect on the feeding behavior of fish, leading to a marked reduction in daily food intake. Ferrari et al. (2011) reported a similar response for juveniles of *Cyprinus carpio* exposed to 0.15 mg Cd/L.

The Fulton's condition factor (K) is an indicator of energy reserves (Smolders et al. 2002). It is usually used as a first approach to evaluate the effect of toxicants on the health status or general condition of an animal (Schlenk et al. 2008). K may reflect the adverse consequences of the pollutant under controlled experimental conditions of food supply. The lower K values of the cadmium-exposed animals (Table 2) would be consistent with the reduction food intake and body weight over time and the consequent energetic balance decompensation.

It is well known that metals such as cadmium, mercury, zinc, chromium and copper are strong inhibitors of olfaction and gustation in fish and that dissolved contaminants can interact with the olfactory neurons as readily as odorants (Tierney et al. 2010). The reduction in fish appetite in our study should be related to the functional impairment of receptor neurons. Likewise, the sensory cells of the lateral line system, which are in constant contact with the aquatic media, might be inhibited by cadmium exposure (Baker and Montgomery 2001). Moreover, fish feeding behavior may also be influenced by both external (e.g., environmental conditions, seasons, hour of day) and internal factors (e.g., hormones such as insulin and leptin, glycemia) (Hoskins and Volkoff 2012; Volkoff 2005, 2009).

The specific metabolic rate (SMR) measured at final exposure time, in the group exposed to 0.8 mg Cd/L showed a significant increase compared to its control (Table 4) which might be due to an increased energy demand. Ferrari et al. (2011) showed a similar trend in *C. carpio* exposed to 0.15 mg Cd/L. They interpreted that fish increased energy demand as a consequence of stress by exposure to the toxicant. In our study, the animals exposed to 0.8 mg Cd/L showed a considerable increase in oxygen consumption measured as SMR and although there was an increasing trend in oxygen extraction efficiency (OEE%), it was not statistically significant.

The ability of fish to extract oxygen from water under stress conditions depends on oxygen affinity to hemoglobin, increased water current through the gills, reduced blood flow, improved diffusion conditions in the gills and/or increased number of lamellae (Zhou et al. 2001). All these adaptive strategies ultimately aim to increase the oxygen supply to the tissues. Espina et al. (2000) observed that grass carps (*Ctenopharyngodon idella*) exposed to 0.5 mg Cd/L for 96 h increased both oxygen consumption and OEE %; similar results were obtained for *C. carpio* (Ferrari et al. 2011). Our results provide evidence that the increase in SMR recorded in *C. decemmaculatus* cannot be attributed to increased oxygen uptake, then, energy storage could be involved.

The amount of ammonia excretion enables the evaluation of nitrogen balance. It is a valuable tool to determine the effects of the environment and nutritional factors on protein metabolism (Uliano et al. 2010; Fournie et al. 2003). The ammonia is the most important excretion product of freshwater fish, representing between 80 and 98% of the total nitrogen excreted (De Boeck et al. 1995). Because most of the nitrogenous end products in freshwater fish originate from protein catabolism, with ammonia as the principal end

product, the contribution of protein catabolism to the total energy production of the fish can be assessed by determination of the ammonia. The ammonia quotient (AQ) estimates the proportion of proteins involved in respiration evaluated as the oxygen consumption rate (De Boeck et al. 1995; Owen et al. 1998), thus clearly representing an index of protein catabolism. An increase in ammonia excretion may be caused by increased protein degradation. However, the ammonia excreted may remain constant or even decrease due to reduced food intake, reduced protein digestibility, or liver function impairment. Damage of the gill epithelium may also obscure the evaluation of ammonia excretion. Therefore, the level of excreted ammonia as a parameter may lead to misinterpretation of results. In our work, no significant differences in ammonia excreted and in AQ were observed between animals exposed to Cd and controls (Table 4).

Scope for growth (SFG) is an integrated physiological parameter; essentially, it is an energy balance equation expressed per individual, which is defined as the energy acquisition from food (assimilation) minus the energy loss from respiration and excretion. In this study, the individuals exposed to 0.8 mg Cd/L showed a significant decrease in SFG (Table 4). The SFG provides a rapid and quantitative assessment of the energy status of animals, as well as insight into the mechanisms of toxicity which may affect changes in growth rate. The physiology of energy balance given by SFG represents the energy available for growth and reproduction of an individual after all physiological demands of respiration and excretion have been met (Scarlet 2015). A reduction of SFG due to stress induced by hypoxia has been preported in *Cyprinus carpio* mainly by a decline in the assimilation efficiency (Zhou et al. 2001), suggesting that this marker is sensitive to and representative of the metabolic status of the fish. In addition, SFG appeared as the most sensitive and useful growth biomarker for cadmium toxicity studies using taxonomically different groups such as marine gastropods (*Nassarius festivus*) and teleosteans (*C. carpio*) (Wo et al. 1999; Ferrari et al. 2011). The SFG is not specific and does not give any information on the stressor, but it can be used to assess the overall status of an organism; it is useful as a measure of individual metabolic performance and provides relevant information at the population level. The analysis of our results showed that cadmium-exposed fish reduced food intake with concomitant decline in the external energy supply from feeding, which was reflected in a decreased assimilation. Like the SFG, which was useful for denoting the overall metabolic status of our test animals, the condition factor K emerged as a sensitive global index of health status.

It is worth to mention that oxygen consumption and ammonia excretion showed variability between assays. Considering that the tested *C. decemmaculatus* were adult, mostly females and came from the same culture, we postulate that these differences may be attributed to a seasonal effect since A1 and A2 were conducted in spring and winter, respectively. In this regard, Menéndez-Helman et al. (2015) also reported seasonal differences in acetylcholinesterase for the same test species.

Finally, this study is the first to report results of energy metabolism for adults of C. *decemmaculatus* under laboratory conditions. These show the deployment of different compensatory mechanisms to counteract stress, in our case caused by toxic cadmium concentrations. Moreover, our results raise the possibility that the metabolic status of the test organisms may affect the response of biomarkers commonly used as endpoints for ecotoxicological evaluations.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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