

# Effects of cadmium on skeletal muscles and swimming performance of *Danio rerio*

C. Agnisola, E. Uliano, P. Simoniello, C.M. Motta\*

Department of Biological Sciences, University of Naples Federico II,  
Via Mezzocannone 8, 80134 Naples, Italy

\* mottacm@unina.it

**KEY WORDS:**  $U_{crit}$ , nitrogen excretion, glycogen distribution, muscle fibers resorbtion

eosin to show general morphology or with periodic acid Schiff (PAS) to highlight glycogen.

## Abstract

**This study describes the physiological and cytological effects of an environmentally realistic concentration of cadmium on muscles organization and swimming performance of *Danio rerio*. Results indicate that the ion reduces the  $U_{crit}$  and that this effect is probably related to the resorbtion of myofibrils observed in red and white muscles.**

## Introduction

Cadmium (Cd) is a widespread contaminant of freshwater ecosystems that is easily uptaken by fish in which exerts highly toxic effects on gills, kidney and liver [1-3]. Cd induces significant alterations also in the muscles [3]; the consequences on swimming performance however have received limited attention. For this reason we treated adults of *Danio rerio* with an environmentally realistic concentration of Cd and determined the effects on the  $U_{crit}$ , the highest sustainable swimming speed achievable by a fish. In parallel muscles morphology and glycogen distribution in fibers were analyzed by cytological techniques.

## Materials and Methods

Adults of *D. rerio* were contaminated with  $CdCl_2$  dissolved in water (0.3 mg/l; Sarno River mouth, [4] and 3 mg/l). Physiological determinations and cytological analyses were carried out 15 and 30 days after the beginning of contamination. Individual  $U_{crit}$  was determined with a swimming tunnel, following a stepwise increase in water speed until fish exhausted [5]. Each step was  $1BL \text{ sec}^{-1}$  high and lasted 3 minutes [6-7]. Waste nitrogen excretion, as an index of protein catabolism, was determined measuring ammonia in water samples using the indophenol method [8] and an Hach spectrophotometer. For cytological analyses muscles sections were stained with haematoxylin-

## Results

The  $U_{crit}$  remains constant or slightly increases during the first 15 days of treatment and then significantly decreases in 0.3 mg/l treated animals (Fig.1 a). Ammonia excretion rate (Fig.1 b) increases significantly during the first two weeks of treatment and returns to the initial value at the end of treatment. Cd causes myofibrils disorganization and partial resorbtion. At both dosages, damages appear in red muscles (Fig.1 d) and extend to white muscles (Fig.1 e, h). At the higher dosage a stretching of the endomysial fibers is also evident (Fig.1 i). This result may be related with a disruption of muscle protein turnover and agrees with the increased ammonia excretion rate in the first half of treatment period. PAS reveals that glycogen content is significantly reduced in fibers treated with 3 mg/l (Fig.1 j-l).

## Discussion

Cd contamination at realistic concentrations significantly reduces swimming performance in *D. rerio*, suggesting that it may have a potentially high ecological impact, by reducing animal fitness. The reduced performance would be correlated with the significant resorbtion of myofibrils observed in red and white muscles. This event is supported by the peak in nitrogen excretion on day 15. Unexpectedly, a weaker negative effect on swim is registered at the higher Cd concentration. This could be explained considering that the higher concentration mobilizes glycogen from fibers making thus available extra energy to maintain a high swimming performance even in presence of cytological damages. In conclusion, cadmium at an environmentally realistic dosage has a toxic effect on muscles fibers thus significantly reducing swimming performance. Consequences at the ecological level are evident.

## References

- [1] Koca S., Koca Y.B., Yildiz S., Gürcü B. 2008. Genotoxic and histopathological effects of water pollution on two fish

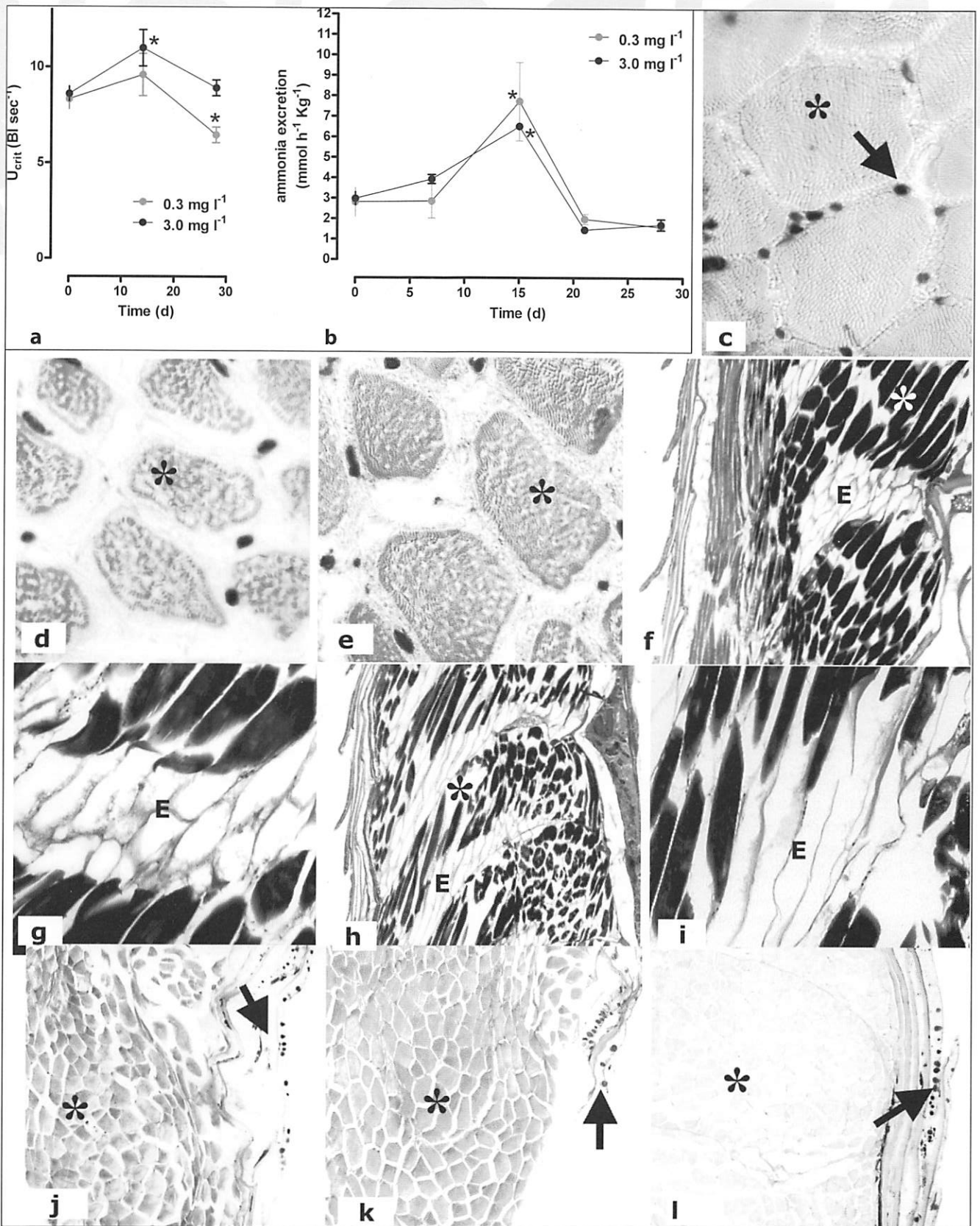


Figure 1. Effects of cadmium on  $U_{crit}$  (a) and ammonia excretion rate (b) in *Danio rerio*. Data are reported as means  $\pm$  SE. t-Student comparison or one way ANOVA followed by Tukey post-hoc test were used as appropriate. (\*: significantly different,  $p < 0.05$ ). (c) untreated animal; muscle fibers with regularly arranged myofibrils (\*). Fiber nuclei (arrow). (d) 0.3 mg/l Cd, 15 days. Red muscle fibers with disorganized myofibrils (\*). (e) 0.3 mg/l Cd, 30 days. White muscle fibers with disorganized myofibrils (\*). (f) untreated animal, frontal section. Muscle fibers are thick, dense and regularly packed (\*); endomysial fibers (E). (g) detail. (h) 3 mg/l Cd, 15 days; frontal section. Muscle fibers are thin and disorganized (\*); the endomysial fibers (E) are stretched. (i): detail. (j-l): PAS staining, cross sections. (j) untreated animal with stained fibers (\*). (k): 0.3 mg/l Cd, 15 days; stained fibers (\*). (l): 3 mg/l Cd, 15 days; unstained fibers (\*). In figures j-l notice the intensely stained mucous cells (arrows).

- species, *Barbus capito pectoralis* and *Chondrostoma nasus* in the Büyük Menderes River, Turkey. Biol. Trace Elem. Res., 122: 276-291.
- [2] Simpson M.G., Parry M., Kleinkauf A., Swarbreck D., Walker P., Leah R.T. 2000. Pathology of the liver, kidney and gonad of flounder impacted by endocrine disrupting chemicals. Mar. Environ. Res., 50: 283-287.
- [3] Koca Y.B., Koca S., Yildiz S., Gürcü B., Osañç E., Tunçbas, O., Aksoy G. 2005. Investigation of histopathological and cytogenetic effects on *Lepomis gibbosus* in the Cine stream with determination of water pollution. Environ. Toxicol., 20: 560-571.
- [4] De Pippo T., Donadio C., Guida M., Petrosino C. 2006. The case of Sarno River (Southern Italy). Effects of geomorphology on the environmental impacts. Environ. Sci. Pollut. Res., 13: 184-191.
- [5] Brett J., Groves T. 1979. Physiological energetics. In: Hoar W.S., Randall D., Brett J.R., *Fish Physiology - Bioenergetics and growth*. Academic Press, New York, 279-352.
- [6] Farlinger, S., Beamish, F.W.H. 1977. Effects of time and velocity increments on the critical swimming speeds of largemouth bass (*Micropterus salmoides*). Trans. Am. Fish. Soc., 106: 436-443.
- [7] Kolok A.S. 1999. Inter individual variation in the prolonged locomotor performance of ectothermic vertebrates: a comparison of fish and herpatofaunal methodologies and a brief review of the fish literature. Can. J. Fish. Aquat. Sci., 56: 700-710.
- [8] Boyd C.E., Tucker C.S. 1992. Water quality and pond soil analysis for aquaculture. Alabama Agricultural Experiment Station, Auburn University, Alabama.