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Energy reserve modification in different age groups of *Daphnia schoedleri* (Anomopoda: Daphniidae) exposed to hexavalent chromium

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ABSTRACT

Caloric content is a reliable biomaker of effect since it is modified by exposure to toxicants that can alter basal metabolism. Since organisms' age modifies how energy resources are allocated and modifies the activity of antioxidant enzymes, the response to toxic agents could be altered with age. Seven age groups of *Daphnia schoedleri* (0, 3, 5, 7, 14, 21, and 28-day-old) were exposed for 24 h to two sublethal concentrations of Cr(VI): 1/25 and 1/5 of the 48 h EC_{50} of each age group, to determine the age at which susceptibility to Cr(VI) is highest. To evaluate energy content, carbohydrate, protein and lipid reserves were quantified and antioxidant enzymes activity was assessed (SOD, CAT, GPx, and GR). Furthermore, an integrative approach was applied to evaluate both sets of responses and interpret them as a whole in a simply visual way, achieved by the integrated biomarker response approach. Results indicate that Cr(VI) induced significant differences in all age groups. Seven and 14-day-old organisms were exposed to the highest concentrations (based on their EC50) and showed greater tolerance to this metal. Susceptibility to the toxicant was highest in younger specimens in which energy requirements are greater due to high growth rates (basal metabolism), as a result of which more energy reserves are expended to satisfy demands in terms of growth and response to toxicants.

1. Introduction

A wide array of assays has been developed to evaluate, with the use of biomarkers, the toxicity induced by contaminants and correlate its effects at organism and sub-organism level with effects at the population level (Villarroel et al., 2009). Biomarkers are defined as changes in biological responses (ranging from molecular through cellular and physiological levels) that indicate deviations from the normal status of an organism resulting from exposure to diverse environmental factors, including xenobiotics (van der Oost et al., 2003).

A potential alternative to traditionally used biomarkers is to assess variation in nonspecific energy components such as glucose, glycogen or lactate, in order to determine variation in stress level or general condition of the organism exposed (Sancho et al., 2009). The stress elicited in organisms by natural causes and/or exposure to toxic agents may induce compensatory mechanisms that alter or modify energy metabolism (De Coen and Janssen, 1997; Bergman-Filho et al., 2011). Since organisms use most of their energy for growth, reproduction, and basal metabolism, higher energy expenditure is required for basal metabolism in order to confront the stress induced by one or a mixture of toxic compounds, thereby eliciting a

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reduction in energy reserves (stored in the form of macromolecules such as lipids and glycogen) (Sancho et al., 2009).

Energy budget assessment can be used as an early warning signal for acute events since its response is usually instantaneous even at low pollutant concentrations (Moolman et al., 2007). Thereafter, changes in organisms' energetic resources are potentially a reliable toxicological tool because a positive net energy balance is needed for maintenance, somatic growth, and reproduction (Verslycke et al., 2003; Smolders et al., 2004).

Widdows and Donkin (1991) stated that the energy resources of organisms represent the integration of various basic body processes such as feeding and nutrition (nutrient uptake and assimilation), respiration, excretion, and reproduction. Variation in the energy content available for growth and reproduction may therefore be useful in evaluating environmental effects, including exposure to different toxic agents (Neuberger-Cywiak et al., 2007). The "metabolic cost" hypothesis suggests toxicant-induced stress elicits metabolic changes that promote a reduction in energy reserves, with adverse effects for growth and reproduction (Calow and Sibly, 1990).

If any stressor disrupts the physiological integrity of organisms, diverse defense and repairing mechanisms will be induced. Nonetheless, these mechanisms depend on energy-requiring processes such as active transport and synthesis activity. As a consequence, dealing with environmental stressors can turn into an energetically costly situation (Smolders et al., 2005).

In cladocerans, protein content and total lipids reflect the general condition of organisms and are reliable indicators of nutritional state (Guisande et al., 1991). Since lipids are the main energy reserve expended by daphnid progeny, it may be useful to evaluate changes in lipid content. In most cases, reduced lipid and protein reserves have been ascribed to an increase in the energy requirements associated with toxicant-induced stress. Exposed organisms obtain from these two reserve components the energy required for detoxification, with consequent loss of these same energy reserves and of structural (protein) components (Sancho et al., 1996). Reduced protein content may be due to hydroxylation of the protein present in different tissues in order to release free amino acids for energy production *via* the Krebs cycle during periods of stress (Begum, 2004).

The basic response of stress in cladocerans enhances the mobilization of carbohydrates, which cover the energy demands in synthesis reactions and provide some intermediaries for various anabolic pathways (De Coen et al., 2001). Moreover, changes in carbohydrate concentrations have been successfully used as stress indicators (Jemec et al., 2007).

Energy content is negatively altered in *Daphnia magna* neonates exposed to different toxicants, as described by Sancho et al. (2009). Moreover, Villarroel et al. (2009) pointed out that the energetic cost of maintenance generates a trade-off in which resources are mainly allocated to survival instead of reproduction. Thereafter, the caloric content assessment can serve as an early indicator of stress not only at a subindividual level but also for inferences at a supraindividual level.

Most studies evaluating energy reserves in cladocerans have focused either on energy resource reallocation to reproduction under normal conditions or on different regimens of caloric intake due to differences in the food ingested (Tessier and Goulden, 1982; Lampart, 1987). Although some studies have evaluated how this energy availability is modified, there is little information on how the content of reserve energy components (carbohydrates, protein, lipids) is modified in organisms of different ages under stress elicited by exposure to environmental toxicants such as Cr(VI), an easily dissolved toxic metal of environmental concern that enters aquatic ecosystems through industrial wastewater discharges (Zayed and Terry, 2003; Lin, 2002) derived from tannery, metal finishing, refractory processes, among others (Domingues et al., 2010).

Chromium acts as a strong oxidizing agent, producing reactive oxygen species (Valko et al., 2005) that lead to alteration in the levels of antioxidant enzymes (Roberts and Oris, 2004; Lushchak et al., 2008). Because of its widespread effects on aquatic biota, Cr(VI) is used as a reference toxicant in the assessment of aquatic toxicity (Martínez-Jerónimo et al., 2006). In recent studies, the effect of age in daphinids' antioxidant activity has been assessed, finding differences among age groups, with a decreasing pattern in this activity that was inversely correlated to age (Arzate-Cárdenas et al., 2011; Arzate-Cárdenas and Martínez-Jerónimo, 2011). Nevertheless, it has not been established if this detoxification process could be energetically costly to cladocerans exposed to Cr(VI) and how age can alter the energetic resources allocation.

Moreover, natural populations have a complex age structure and their dynamics can be modified by natural or anthropogenic stressors. The successful survival of different age groups may be related to their susceptibility to environmental stressors, and neonates are expected to be the most sensitive age group (Hoang and Klaine, 2007). Therefore, the aim of the present study was to evaluate in different age groups of the cladoceran *Daphnia schoedleri* the extent to which exposure to Cr(VI) modifies energy reserves (determined by carbohydrate, protein and lipid amounts as well as caloric content) and antioxidant enzyme activity, both of them assessed through the integrated biomarker response approach described by Beliaeff and Burgeot (2002). *D. schoedleri* is a zooplanktonic cladoceran with a cosmopolitan distribution, including different localities in Mexico (Elías-Gutiérrez et al., 2008).

2. Materials and methods

The *D. schoedleri* strain used in the present study was obtained from the cladoceran collection of the Experimental Hydrobiology Laboratory (Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional). This strain has been maintained active for more than 15 years. Test specimens were obtained from controlled cultures of known age, grown in 500 mL containers with 400 mL ISO medium (ISO, 1982) (reconstituted hard water with the same ionic concentration as the formulation proposed by USEPA, 2002). As described in Arzate-Cárdenas and Martínez-Jerónimo (2012), 10 parthenogenetic females were placed in each container and fed the microalga *Pseudokirchneriella subcapitata* at 6×10^5 cells mL^{-1} (Martínez-Jerónimo et al., 2008). These cultures of breeding females were maintained at $25 \pm 1\,^{\circ}C$, with a 16 h:8 h (light:dark)