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# Carbofuran-induced alterations in biochemical composition, lipoperoxidation, and $Na^+/K^+$ATPase activity of *Hyalella pleoacuta* and *Hyalella curvispina* in bioassays

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## Abstract

The present study investigated the effects of carbofuran on the energy metabolism (levels of glycogen, total proteins, total lipids, triglycerides, and lipoperoxidation), $Na^+/K^+$ATPase activity, and reproductive parameters (formation of couples, ovigerous females, and mean number of eggs) in the freshwater amphipods *Hyalella pleoacuta* and *Hyalella curvispina*. These crustaceans live in limnetic environments of the plateau (*H. pleoacuta*) and coastal plain (*H. curvispina*) of the state of Rio Grande do Sul in southern Brazil. The animals were collected in the winter of 2006 in the Vale das Trutas (28°47′00″S–49°50′53″W) in the Municipality of São José dos Ausentes, and in Gentil Lagoon (29°56′30″S, 50°07′50″W) in the Municipality of Tramandaí. In the laboratory, the amphipods were kept submerged in aquariums under controlled conditions of photoperiod (12 h light: 12 h dark), temperature (23 °C ± 1), and constant oxygenation. Animals were exposed to carbofuran at a dose of 5 or 50 μg/L for a period of 7 days. At the end of this period, the animals were immediately frozen for determination of the biochemical parameters, lipoperoxidation levels (TBARS), and enzyme $Na^+/K^+$ATPase activity. During each day of culture, several reproductive parameters were observed. Statistical analysis (ANOVA) revealed that carbofuran induces significant decreases in glycogen, proteins, lipids, triglycerides, and $Na^+/K^+$ATPase, as well as a significant increase in lipoperoxidation levels. Studies of all the biochemical parameters seem to be quite promising, in order to assess and predict the effects of toxicants on non-target organisms. The results also suggest that the reproductive parameters (formation of couples, ovigerous females and mean number of eggs) may provide sensitive criteria for assessing ecotoxicological effects. Furthermore, *H. pleoacuta* and *H. curvispina* are suitable organisms for use in toxicity tests, and we suggest that they are sensitive species that could be used in monitoring studies.


*Keywords:* *Hyalella pleoacuta*; *Hyalella curvispina*; Intermediate metabolism; Lipoperoxidation; $Na^+/K^+$ATPase; Toxicology bioassay

## 1. Introduction

Amphipods of the genus *Hyalella* are common in the Nearctic and Neotropical regions, with 45 described species (González and Watling, 2001; González et al., 2006). They are found in a variety of freshwater habitats, such as permanent reservoirs, lakes, impoundments, and streams, and often cling to the vegetation, swim in the water, or burrow in the sediment, where they are important members of the benthic fauna (Kruschwitz, 1978; Wellborn, 1995; Grosso and Peralta, 1999).

Recently, a great deal of attention has been devoted to the use of physiological and energetic processes of non-target organisms (e.g., feeding parameters, growth, respiration, reproduction, and energy allocation mechanisms) as sensitive indicators of toxic stress from exposure to metals (Drobne and Hopkin, 1994; Khalil et al., 1995) and chemicals, including pesticides (Mohamed et al., 1992; Van Brummelen et al., 1996). The ecological relevance of these parameters is clear, because short-term exposures can have long-term effects on the life cycles of

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non-target organisms, even though some compounds do not persist for long in the soil (Ribeiro et al., 2001).

Disturbance of the homeostasis of an organism leads to compensatory, adaptive and finally pathological processes which are mostly energy-demanding. Therefore, the metabolic rate of an organism must increase under toxic stress (Calow and Sibly, 1990). Because the energy resources of organisms are limited, the additional metabolic costs result in a reallocation of energy resources, and can only be met at the expense of other energy-demanding processes (Beyers et al., 1999) or by increased energy intake.

Carbofuran is a carbamate nematicide and insecticide. Like other carbamate and organophosphate pesticides, carbofuran inhibits acetylcholinesterase (AChE) (Kuhr and Dorough, 1976; US EPA, 1988). In invertebrates, AChE inhibitors act on the central nervous system (Eldefrawi, 1985). AChE inhibition in insects causes hyperactivity, loss of coordination, convulsions, paralysis, and death (Kuhr and Dorough, 1976). Consequently, poisoned organisms experiencing loss of coordination or orientation may be additionally compromised while coping with environmental stresses, such as suspended solids, that require changes in physical activity. Increased metabolic activity (e.g., Samson et al., 1984) coupled with increased physical stress may also deplete energy stores in an organism (e.g., Rambabu and Rao, 1994). If energy stores or physical activity are affected by exposure to both carbofuran and suspended solids, and energy availability affects the toxic response of *D. magna*, both stressors could affect similar physiological processes. Therefore, it is reasonable to hypothesize that the toxic effects of carbofuran and suspended solids could combine in an additive manner.

The toxicity of carbofuran to aquatic life has been reviewed by Elser and Kimmel (1985) and by the Canadian Water Quality Guidelines (Anonymous, 1989). $LC_{50}$ (24–48 h) values for fish range between 280 and 8500 μg/L (Hejduk and Svobodova, 1980; Bakthavathsalan and Reddy, 1982; Stephenson et al., 1984), while amphibians, mollusks, oligochaetes, higher plants, and algae are generally less sensitive (Dad et al., 1982; Hartman and Martin, 1985; Khangarot et al., 1985). In contrast, crustaceans and insect larvae are among the most susceptible groups of organisms, with acute toxicities in the range of 1.6–500 μg/L (Hartman and Martin, 1985; Johnson, 1986; Parsons and Surgeoner, 1991).

ATPases play important roles in intracellular functions and in all types of physiological activity. $Na^+/K^+$ATPase is a membrane-bound enzyme found in animal cells; its most important feature is the coupling of the free energy stored within the ATP molecule to the translocation of $Na^+$ ions. Often, ATPase activity is used as a sensitive indicator of heavy-metal toxicity (Haya and Waiwood, 1983), although there is evidence that organic pollutants can inhibit ATPase activity in concentration-based experiments (Reddy et al., 1992).

Some studies have reported that the peroxidation of membrane phospholipids induced by reactive oxygen species and/or free radicals leads to alterations in the membrane structure and functions (Vercesi et al., 1997; Milatovic et al., 2005). These degenerative changes can affect dynamic properties of the membranes such as fluidity and permeability, and consequently the activity of various membrane-associated enzymes (Mecocci et al., 1997). Several investigators have reported that lipid peroxidation products disrupt neuronal ion homeostasis by impairing the function of membrane-bound ion-motive ATPases such as $Na^+/K^+$ATPase (Keller et al., 1997; Mark et al., 1997).

The aims of the present study were to quantify biochemical responses of ecological importance (energy reserves, lipid peroxidation, and $Na^+/K^+$ATPase activity, as well as reproductive parameters) in the organisms exposed to carbofuran; and to relate data obtained for the biochemical parameters, to the physiological status of the animals. The purpose was to obtain basic physiological data to support the development of a new model for toxicology tests in the laboratory, because the number of species used in standard toxicology tests is quite limited, and almost none of the species is native to Brazil. The reason for this small number of available species is that most toxicology tests require continuous cultures to provide sufficient numbers of organisms. This rule has sharply limited the choice of species that can be used (Brendonck and Persoone, 1993).

## 2. Material and methods

The animals were cared for in accordance with Brazilian laws. The animals were used with the permission of the Ethics Committee of the Pontifícia Universidade Católica do Rio Grande do Sul (License 0004/03).

### 2.1. Collection of Hyallela pleoacuta and Hyalella curvispina

In order to establish the profile of variation in the biochemical composition, lipid peroxidation levels and levels of activity of $Na^+/K^+$ATPase in the amphipods, *H. curvispina* and *H. pleoacuta*, submitted to two concentrations of carbofuran (5 or 50 μg/L). The collections were made in the winter (June, July and August). The animals were collected together with macrophytes from each locale (*H. curvispina* with *Salvinia biloba*, and *H. pleoacuta* with *Callitriche rimosa*). *H. curvispina* (120 males and females) were collected in Gentil Lagoon (29°56′30″S, 50°07′50″W), in Tramandaí; and *H. pleoacuta* (120 males and females) in São José dos Ausentes Municipality (28°47′00″S–49°50′53″W), Rio Grande do Sul, Brazil. The amphipods were collected by means of fish traps and bottom grabs, and were transported on ice in insulated containers to the Laboratório de Fisiologia da Conservação of PUCRS. Twenty animals of each sex and each species were cryoanesthetized, in order to assess and compare whether there were any differences, if any, between the animals collected in the wild and the animals that received artificial diets and were exposed to the pesticide.

To characterize the collection locales, the pH, temperature, and hardness of the water were measured during the months of collection. The pH was determined with a portable pH meter, and the water temperature with an internal-scale thermometer. The hardness was determined using a classic method of volumetric complexation (Adad, 1982). The mean temperatures