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Seasonal variations of the energy metabolism of two sympatric species of *Hyalella* (Crustacea, Amphipoda, Dogielinotidae) in the southern Brazilian highlands

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Abstract

Aquatic organisms exist in a constantly fluctuating habitat, with changes in photoperiod, temperature, pH, dissolved organic content, dissolved oxygen and food supply. Organisms must alter past of their physiological and biochemical processes to cope with these changes. We compared the effect of seasonal variations in factors related to energy metabolism of two species of sympatric amphipods, *Hyalella pleoacuta* and *Hyalella castroi*. The animals were collected monthly from April 2004 through March 2006. Contents of glycogen, proteins, lipids, triglycerides and the levels of lipoperoxidation were determined in males and females throughout the year by using spectrophotometric methods. Observations revealed significant seasonal differences in biochemical composition, as well as differences among sexes and species. Environmental conditions (e.g., trophic conditions) and reproduction are supposed to be the main processes influencing the seasonal patterns of variation in biochemical composition. Both species of *Hyalella* show ecological and behavioral differences, especially by resources such as food, space and seasonal variations of energy metabolism, which might facilitate their coexistence in the same habitat.

Keywords: Crustacea; Amphipod; *Hyalella pleoacuta*; *Hyalella castroi*; Energy metabolism; Lipoperoxidation; Seasonality; Sympatric

1. Introduction

Members of the genus *Hyalella* are common in the Nearctic and Neotropical regions, with 45 described species (Gonzáles and Watling, 2001). They are found in a variety of freshwater habitats, such as permanent reservoirs, lakes, ponds and streams, and often clinging to the vegetation, swimming in the water, or burrowing in the sediment (Kruschwitz, 1978; Wellborn, 1995; Grosso and Peralta, 1999). Amphipods are important members of their communities; they are detritivorous and herbivorous, and serve as food for many other organisms (Schmitt, 1965; Muskó, 1993; Pilgrim and Burt, 1993). Therefore amphipods constitute important links in the food web, serving to transfer energy from algae and macrophytes to higher-level consumers (Moore, 1975; Vassillenko, 1991; Pilgrim and Burt, 1993).

Aquatic organisms exist in a constantly fluctuating habit, with changes in photoperiod, temperature, pH, dissolved organic content, dissolved oxygen and food supply (Reid and Wood, 1976). Organisms must alter part of their physiological and biochemical processes in order to cope with these changes (Hochachka and Sommero, 1984). Nutritional deprivation is a natural part of the life cycle of many aquatic organisms, as the result of winter torpor, seasonal elimination of a food source or behavioral modifications during mating and spawning (Schirf et al., 1987).

Studies of the intermediate metabolism in crustaceans have revealed great inter- and intra-species variability, which has

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Table 1
Seasonal variations of the oxygen content (mg/L), pH, and water temperature (°C) and air temperature (°C) in the natural environment

	Spring	Summer	Autumn	Winter
Oxygen content (mg/L)	7.18±0.07	6.26±0.34	4.88±0.65	7.24±0.07
pH	7.04±0.05	6.87±0.04	7.09±0.21	6.38±0.26
Water temperature (°C)	18.27±1.03	19.80±0.59	15.48±1.04	11.03±1.18
Air temperature (°C)	18.93±1.98	22.73±1.71	17.73±1.49	10.37±0.55

Results represent the mean±standard error of the mean.

made it difficult to establish a standard metabolic profile. Without considering differences among the biochemical methods used by various investigators, this variability can be attributed to multiple factors, such as habitat (terrestrial, marine, estuarine, or freshwater), period of life cycle, sexual maturity (especially in females), nutritional state, available food, and seasonality, because all these factors lead to different patterns of metabolic responses (Chang and O'Connor, 1983; Kucharski and Da Silva, 1991a,b; Oliveira et al., 2003; Rosa and Nunes, 2003b; Ferreira et al., 2005).

Crustaceans contain high concentrations of lipids, although they have no differentiated adipose tissue but store lipids mainly in muscle tissue and the hepatopancreas (O'Connor and Gilbert, 1968; Chang and O'Connor, 1983; Herreid and Full, 1988; Kucharski and Da Silva, 1991a). During periods of high energy demand, such as molting and gametogenesis, large amounts of lipids are mobilized, especially from the hepatopancreas (Kucharski and Da Silva, 1991a; Quigley et al., 1992; Rosa and Nunes, 2003b; Oliveira et al., in press).

In crustaceans, glycogen is stored mainly in the muscles, hepatopancreas, gills, and hemocytes; however, the storage locations vary among different species (Parvathy, 1971; Johnston and Davies, 1972; Herreid and Full, 1988). The stored glycogen is used in the processes of change, hypoxia and/or anoxia, osmoregulation, growth, different periods during reproduction, and during periods of starvation (Chang and O'Connor, 1983; Kucharski and Da Silva, 1991a,b; Rosa and Nunes, 2003a; Oliveira et al., 2001, 2004).

Some studies have reported variations in protein content during ovarian development in crustaceans. These variations may result from an increase in the biosynthesis of various proteins, including hormones, enzymes, and lipoproteins involved in gonadal maturation (Rosa and Nunes, 2003a; Yehezkel et al., 2000). The muscle is apparently the main protein-storage location in crustaceans. Proteins are a structural, functional and energy constituent of tissues and play an important role in spawning, fertilization and normal development of embryo in decapods (Garcia et al., 2003; Rosa and Nunes, 2003a,b; Rodriguez-González et al., 2006). Other studies have demonstrated variation in protein content during periods of starvation (Yehezkel et al., 2000).

Malondialdehyde, a breakdown product of lipid endoperoxides, is an expression of lipid peroxidation and has been used with success in aquatic invertebrates as a general indicator of toxicant stress derived from various types of contamination (Zwart et al., 1999; Livingstone, 2001; Wilhelm et al., 2001; Timofeyev et al., 2006, 2007). Neuparth et al. (2005) described that in *Gammarus locusta* maintained with sediments have high levels of organic matter content present higher levels of lipoperoxidation. Effectively, some authors agree that endogenous variables like nutritional status, age, sex, growth and reproduction influence the peroxidation status of organisms (Viarengo et al., 1991; Correia et al., 2002, 2003).

The aim of the present study was to characterize the seasonal variations in the intermediate metabolism (total lipids, triglycerides, proteins, and glycogen) and in the levels of lipoperoxidation in two sympatric species of hyalellids in the natural environment. We also investigated possible relationships between biochemical, biological and ecological parameters to comprehend the sympatric relation in these animals.

2. Material and methods

The animals were cared for in accordance with guidelines such as the Guide for the Care and Use of Laboratory Animal (1996, published by National Academy Press, 2101 Constitution Ave. NW, Washington, DC 20055, USA). The animal were used with the permission of the Ethic Committee of the Pontifícia Universidade Católica do Rio Grande do Sul (License 0004/03).

2.1. Collection of H. pleoacuta and H. castroi

To establish the profile of seasonal variation, the collections were initiated in April 2004 and continued through March 2006. Thirty individuals of each species (fifteen males and fifteen females), *H. pleoacuta* (González, Bond-Buckup and Araújo, 2006) and *H. castroi* (González et al., 2006) were collected monthly, always at the same time of day (1 pm until

Table 2
Seasonal concentration of glycogen in females and males of *Hyalella pleoacuta* and *Hyalella castroi* collected in the natural environment

	Spring	Summer	Autumn	Winter
Hyalella pleoacuta				
Females#	0.772±0.128^{a}	2.326±0.649^{b}	8.032±0.982^{abc}	3.325±0.677^{c}
Males#	1.815±.228^{a}	1.826±0.255^{b}	8.764±3.169^{abc}	1.771±0.322^{c}
*Hyalella castroi**				
Females	1.376±0.17^{b}	0.594±0.006^{c}	0.432±0.049^{a}	2.925±0.516^{abc}
Males	0.661±0.05^{c}	0.177±0.022^{bd}	1.121±0.183^{ab}	3.280±0.405^{acd}

All results represent the mean±standard error of the mean, and are expressed in mmol/g. The number of animals represented by each point was 30. The same letter represents significant difference between the seasons. # Significant difference between species. * Significant difference between sexes.