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Comparative Biochemistry and Physiology, Part A 149 (2008) 59–67
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www.elsevier.com/locate/cbpa

Seasonal variations in the biochemical composition of the crayfish *Parastacus defossus* (Crustacea, Decapoda) in its natural environment

L. Buckup [c], B.K. Dutra [a], F.P. Ribarcki [b], F.A. Fernandes [a], C.K. Noro [c], G.T. Oliveira [a], A.S. Vinagre [b,*]

[a] *Departamento de Ciências Morfofisiológicas – Programa de Pós-graduação em Zoologia - Laboratório de Fisiologia da Conservação - Faculdade de Biociências da Pontifícia Universidade Católica do Rio Grande do Sul, Brazil*
[b] *Laboratório de Histofisiologia Animal – Curso de Biologia - Universidade Luterana do Brasil, Av. Farroupilha, 8001, CEP 92450-900, Canoas/RS, Brazil*
[c] *Departamento de Zoologia – Programa de Pós-graduação em Biologia Animal - Instituto de Biociências - Universidade Federal do Rio Grande do Sul, Brazil*

Received 3 September 2007; received in revised form 10 October 2007; accepted 10 October 2007
Available online 18 October 2007

Abstract

The crayfish *Parastacus defossus* occurs in Uruguay and the state of Rio Grande do Sul in Brazil. It lives in swamps and lakes, where it digs sloped subterranean tunnels that are used as burrows. Because there is little information about the biology, physiology and ecology of this species, the aim of this study was to identify the seasonal variations of its carbohydrate and lipid metabolism. Crayfish were collected monthly (from November 2002 to February 2004) in the Lami region, Porto Alegre municipality (30°11′41″S — 51°06′00″W). Haemolymph samples, used for determination of glucose, total proteins, triglycerides, total cholesterol and total lipids, were collected in the field using potassium oxalate as an anti-clotting agent. The animals and haemolymph samples were immediately frozen in the field. In the laboratory, the hepatopancreas, gills and abdominal muscles were removed for determination of glycogen, triglycerides, total cholesterol and total lipids. The findings suggest that in *P. defossus*, lipids are an important reserve of energy used during reproduction in both males and females; whereas glycogen may be used during periods of intense activity or environmental stress.

Keywords: Parastacidae; Crayfish; *Parastacus defossus*; Biochemical composition; Carbohydrate metabolism; Proteins; Lipid metabolism; Seasonality

1. Introduction

Crustaceans are exposed to a large number of environmental variables, which follow annual and daily cycles according to the geographical region, and which affect their behavior, feeding and metabolism. Study of the intermediate metabolism in crustaceans has revealed the existence of wide inter- and intra-specific variability, which makes it difficult to determine a standard metabolic profile (Oliveira et al., 2003). This variability can occur because of multiple factors, such as habitat, stage in the molt cycle, sexual maturity (especially in females), feeding state, available food, and seasonality (Schirf et al., 1987; Kucharski and Da Silva, 1991b).

Glucose is the principal monosaccharide present in the haemolymph of crustaceans, and it serves seven main purposes: synthesis of mucopolysaccharides, synthesis of chitin, synthesis of ribose and nicotinamide adenine dinucleotide phosphate reduced (NADPH), formation of pyruvate, synthesis of glycogen and an energy source (Chang and O'Connor, 1983; Herreid and Full, 1988). Stable glucose haemolymph levels are essential for the regular functioning of the nervous, muscle and reproductive systems. Glucose can be accumulated in the form of glycogen in the hepatopancreas and in other tissues, such as the muscles and the gills (Chang and O'Connor, 1983; Loret et al., 1989; Loret and Devos, 1992; Vinagre and Da Silva, 1992; Schmidt and Santos, 1993; Oliveira and Da Silva, 1997; Vinagre and Da Silva, 2002; Oliveira et al., 2003; Marqueze et al., submitted for publication).

The storage-mobilization cycle of glycogen, and the haemolymph glucose reserves vary widely, and depend, with other factors, on the molt stage, season, diet, nutritional state,

* Corresponding author. Tel.: +55 51 477 9104; fax: +55 51 477 4000.
E-mail address: apvinagre@uol.com.br (A.S. Vinagre).

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doi:10.1016/j.cbpa.2007.10.008

circadian cycle, salinity and dissolved oxygen (Chang and O'Connor, 1983; Herreid and Full, 1988; Loret et al., 1989; Vinagre and Da Silva, 1992; Kucharski and Da Silva, 1991a,b; Morris and Airriess, 1998; Oliveira et al., 2004). In *Aegla ligulata*, males have higher contents of glucose in the haemolymph than females during summer; but in spring, both sexes show hyperglycemia compared with other seasons (Oliveira et al., 2003). In males of *Ocypode quadrata*, the highest glucose levels in haemolymph occur during fall (Vinagre et al., 2007).

In the absence of adipose tissue in crustaceans, the hepatopancreas seems to be the main site of lipid storage (O'Connor and Gilbert, 1968; Chang and O'Connor, 1983; Herreid and Full, 1988; Kucharski and Da Silva, 1991a; Muriana et al., 1993; Garcia et al., 2002), although lipids can also be accumulated in muscle tissue and in the female gonad (Komatsu and Ando, 1992). In the estuarine crab *Chasmagnathus granulatus* (Dana, 1851), for example, Kucharski and Da Silva (1991b) found that total lipids represent more than 20% of the weight of the hepatopancreas.

Several studies have demonstrated that during periods of high energy demand, such as molting and gametogenesis, there is a pronounced degradation of lipids, especially those stored in the hepatopancreas, as observed by Kucharski and Da Silva (1991a) in *C. granulatus*. Rosa and Nunes (2003b) observed a significant increase in the levels of total lipids and cholesterol in gonadal tissue of *Aristeus antennatus*, *Parapenaeus longirostris* and *Nephrops norvegicus* from the Portugal coast. This increase may be related to the stage of ovary maturation.

The muscle is apparently the main protein-storage location in crustaceans. In decapods, the free amino acids in the tissues reach levels tenfold higher than those observed in vertebrates. Several studies suggest that these amino acids participate in osmoregulation and in the control of cell volume (Gilles, 1982; Chang and O'Connor, 1983; Schein et al., 2004). Other studies have demonstrated variation in protein content during ovarian development in crustaceans. These variations may result from increased biosynthesis of several proteins, including enzymes, hormones and lipoproteins involved in gonadal maturation (Yehezkel et al., 2000; Rosa and Nunes, 2003a,b).

According to López-Greco and Rodríguez (1999), the beginning of reproduction is a critical event in the life history of animals, and is related to reproductive effort, defined as the proportion of body energy transferred to reproduction. Analysis of biochemical composition and its seasonal variations is important for reproductive biology, because it is fundamental to understand how different organs can act to store and transfer organic reserves to support gonadal maturation, reproductive period and the maintenance of the animal (Pillay and Nair, 1973; Rosa and Nunes, 2003a). Studies of the reproductive biology of members of the genus *Parastacus* are few. In Brazil, studies have been carried out with *Parastacus defossus* (Almeida and Buckup, 1999), *Parastacus brasiliensis* (Fontoura and Buckup, 1989b; Almeida and Buckup, 1997, 2000) and *Parastacus varicosus* (Castiglioni et al., 2007).

The family Parastacidae is represented in South America by the genera *Parastacus*, *Samastacus* and *Virilastacus*. Only members of *Parastacus* occur in Brazil, preferentially in marshy lentic environments on the plains, and in small, slowly flowing streams (Buckup and Rossi, 1980; Fries, 1980; Fontoura and Buckup, 1989a). Most of the species, including *P. defossus*, construct underground habitations in the form of simple or branched tunnels that reach groundwater level and have one or more openings on the surface. The animals are nocturnal, when they leave their burrows to hunt for food in or near the water (Buckup, 1999). Noro (2007), studying the gastric contents of *P. defossus*, found that, like other parastacids, this species is an opportunistic omnivore with a diet based on plants. The reproduction of this species begins in winter and peaks in spring (Noro, 2007).

In comparison with the extensive literature about biochemistry and reproductive aspects of marine and estuarine crustaceans, fewer studies have considered freshwater crustaceans. The objective of the present study was to evaluate, in the natural environment, the effect of seasonal variations on the biochemical composition of the freshwater crayfish *P. defossus*. The purpose was to obtain basic physiological data to support adequate conservation of these populations.

2. Materials and methods

The animals were cared for in accordance with Brazilian laws, and were used with the permission of the Ethics Committee of the Pontifícia Universidade Católica do Rio Grande do Sul (License 0002/03).

2.1. Sampling

Crayfish (*P. defossus* (Crustacea, Decapoda, Parastacidae) were collected monthly (November 2002 to February 2004) in the Lami region, Porto Alegre municipality (30°11′41″S — 51°06′00″W). Twenty adults of *P. defossus* in stage C or D of the intermolt cycle (Drach and Tchernigovtzeff, 1967) were collected in each season from the Guaíba estuary.

Haemolymph samples were collected (0.8 mL) in the field, by puncturing the membrane at the base of the chelipeds and pereiopods with a syringe containing 10% potassium oxalate as an anti-clotting agent. The samples and the animals were transported to the laboratory (Laboratório de Carcinologia of the Universidade Federal do Rio Grande do Sul) in insulated containers with ice (4 °C). In the laboratory, the crayfish were separated by sex, weighed on an electronic balance (0.001 g), and numbered. The animals were then frozen for later extraction of the main metabolite storage tissues (hepatopancreas, gills and abdominal muscle) and gonadal analysis to confirm their sex.

2.2. Biochemical assays

2.2.1. Haemolymph measurements

The metabolic parameters of the haemolymph sample of each animal were determined in triplicate using spectrophotometric methods.

Glucose levels were measured by the glucose-oxidase method, using a Bioclin Kit (glucose GOD-CLIN). Results are expressed in mmol/L.