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NOTE

Effects of metacercariae (Digenea: Microphallidae) on the hepatopancreas of *Chasmagnathus granulata* (Decapoda: Grapsidae)

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ABSTRACT: We analysed the effects of infection by *Microphallus szidati* metacercariae on carbohydrate (glucose and glycogen) levels and histopathology of the hepatopancreas of the estuarine crab *Chasmagnathus granulata*. No significant differences in infection were found between the left and right sides of the hepatopancreas, and infection intensity ranged from 2 to 802. The mean intensity of metacercariae cysts was 54.79 ± 12.64 (±1 SE) with a prevalence of 90.1%. Hepatopancreatic glycogen was higher in infected crabs (28.2 ± 2.2 mg g^{-1} tissue) than in non-infected ones (21.7 ± 1.9 mg g^{-1} tissue). Further, high intensities of infection (>6 parasites per tissue section) promoted necrosis of the hepatopancreatic tubules and hemocytic infiltration around the cysts. Thus, the presence of *M. szidati* metacercariae in the hepatopancreas of *C. granulata* can cause structural damage to the tissue, altering its glycogen levels, and can be considered as a potential physiological stress factor

KEY WORDS: Parasitism · Carbohydrates · Crab · Hepatopancreas · Physiology

The estuarine crab *Chasmagnathus granulata* (Decapoda, Grapsidae) inhabits salt marshes from Brazil to Argentina (Boschi 1964) and has been employed in toxicological (Monserrat et al. 1996) and physiological studies (Santos & Colares 1986). In Argentina, the parasite *Microphallus szidati* was recorded in the grapsid crabs *Cyrtograpsus angulatus* and *C. granulata*, and it was suggested that they were the secondary intermediate hosts of this parasite (Martorelli 1986a, b, 1989).

In *Chasmagnathus granulata*, a hyperglycemic response was observed when the crab was exposed to atmospheric air (Santos & Colares 1986), osmotic shock (Nery & Santos 1993) or to pollutants (Monserrat et al. 1996). In addition to physico-chemical parameters, however, health status may also be a stress factor (Grizzle 1981). Considering that the hepatopancreas is the primary organ involved in carbohydrate metabolism of crustaceans (Chang & O'Connor 1983), the presence of *Microphallus szidati* metacercariae in the hepatopancreas of *C. granulata* could be a physiological stress factor. Therefore, we evaluated the presence of *M. szidati* in the hepatopancreas of *C. granulata* from Lagoa dos Patos estuary (Southern Brazil), the histopathology associated with infection in this organ, and the possible influence of infection intensity on glucose and glycogen levels.

Material and methods. Adult male crabs were captured from salt marshes near Rio Grande-RS (Southern Brazil) and transferred to the laboratory, where they were acclimated for at least 15 d in 250 l tanks containing sea water at 20 ppt salinity, and 20 ± 2°C. Three days a week, the crabs were fed ground beef *ad libitum*.

The metacercariae used for parasite identification were excysted spontaneously from dissected hepatopancreatic tissue in a saline medium (NaCl 0.75%) for 1 to 2 h at 37°C. The emerged metacercariae were fixed in AFA and stained in Semichon's carmine and Gomori's trichromic. They were then morphologically compared to the paratypes of Martorelli (1986a).

For the 2 following experiments, crabs were weighed and measured, cryoanesthetized, and sacrificed by cutting their cephalothorax into 2 equal halves (left and right). The mean number (±1 SE) of parasites found in the left half of the hepatopancreas (14.6 ± 6.4) was not significantly different from that found in the right half (16.6 ± 7.8) ($p > 0.05$; paired sign test; $n = 11$).

In the first experiment, 51 adult male crabs (mean weight ±1 SE = 9.79 ± 0.29 g; mean carapace width ±1 SE = 2.49 ± 0.02 cm) were employed. One half of the hepatopancreas was dissected in order to estimate the number of parasite cysts. The isolation was performed after tissue digestion for 1 to 2 h at 37°C in 1% pepsin: 0.5% HCl. The cysts were collected from the resultant digest using a 150 µm sieve mesh. Counts were per-

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formed using a stereoscopic microscope (20×). The other half of the hepatopancreas was used for glucose and glycogen determinations. This was achieved by homogenizing the tissue in 0.1 M sodium citrate, pH 5.0. Determinations were done as described by Nery & Santos (1993). The glucose concentration was adjusted to the tissue weight of each sample. The total wet weight of the hepatopancreas was estimated using the figure of 3.2% of the total wet weight of this species as determined by Bianchini et al. (1990).

Correlations between the number of cysts/hepatopancreas and total body weight, carapace width, and glucose and glycogen content were analyzed employing the non-parametric Spearman correlation. The quartiles of the parasite count distribution were employed to define 4 infection categories (0–6; 7–18; 19–46; and >46 cysts/hepatopancreas). Glucose and glycogen content of hepatopancreatic tissue for the 4 infection categories were analyzed using an ANOVA to assess possible significant differences between categories ($p < 0.05$).

Error in the method depicted by Nery & Santos (1993) for carbohydrate determinations was analyzed by means of the variation coefficient after measuring, in triplicate, glucose or glycogen content of standard solutions. The variability coefficient of glucose and glycogen concentrations of the hepatopancreas was estimated after subtraction of the glucose and glycogen contents of the parasites. The carbohydrate content of the parasites was determined using 26 pools of 80 cysts. They were isolated and homogenized in 2 ml of 0.1 M sodium citrate, pH 5.0. Carbohydrate contents were determined as already described for the hepatopancreatic tissue and the mean glucose and glycogen content per cyst was then calculated. Parasite carbohydrate content was estimated taking into account the number of parasites present in the hepatopancreas.

The lipid content of the cysts was also determined. Homogenates were prepared using a chloroform/ethanol mixture (2:1). A commercial reaction kit (Total Lipids®, Labtest Diagnostica SA, Goiânia-GO, Brazil) was employed to determine the total lipid content in 15 pools of 80 cysts.

In the second experiment, 15 adult male crabs (mean weight ±1 SE = 8.34 ± 0.29 g; mean carapace width ±1 SE = 2.39 ± 0.02 cm) were employed to analyze the relationship between frequency of cysts in the hepatopancreas and tissue damage. One half of the hepatopancreas was dissected, fixed in Bouin's solution for 24 h, transferred to 70% ethanol, dehydrated and embedded in paraffin. Tissues were sectioned at 6 µm thickness and stained with Harris' hematoxylin-eosin. The tissue slides were examined, and pathological processes, evidenced by delamination of the hepatopancreatic epithelium, were registered.

Results. The metacercariae found in the hepatopancreas of *Chasmagnathus granulata* (Fig. 1) were morphologically identical to the paratypes of *Microphallus szidati* Martorelli (1986a). The infection intensity ranged from 2 to 802 metarcercariae/complete hepatopancreas, while mean relative density (metacercariae per hepatopancreas) was 54.8 ± 12.6. The mean infection intensity was 60.8 ± 13.2 metacercariae/infected hepatopancreas, while the prevalence was 90.1% ($n = 81$). A significant ($p < 0.01$) correlation was found between the estimated number of parasites and weight (Spearman correlation: 0.30, $n = 81$) or carapace width of infected crabs (Spearman correlation: 0.29, $n = 81$).

No significant differences ($p > 0.05$) in either glucose or glycogen content in the hepatopancreas were detected among the 4 infection levels analyzed. Nor was there any significant correlation between the estimated number of parasites and glucose ($r = -0.05$; $p = 0.76$; $n = 39$) or glycogen ($r = 0.29$; $p = 0.08$; $n = 39$) content. Despite the high content of glycogen and the lack of correlation with the number of parasites, the mean glycogen content of infected crabs was significantly higher (28.2 ± 2.2 mg g^{-1} tissue; $n = 34$) than that observed for non-infected crabs (21.7 ± 1.9 mg g^{-1} tissue; $n = 5$) (t-test with separate variance estimates; $p = 0.04$). No significant difference was found in glucose levels of infected (13.9 ± 0.8 mg g^{-1} tissue; $n = 34$) and uninfected crabs (16.4 ± 3.5 mg g^{-1} tissue; $n = 5$) (t-test; $p = 0.34$).

The error introduced by the parasites in the glucose determination (1.0%) was similar to that of the glucose determination method itself (2.1%). The error introduced by parasites in glycogen determination (6.4%)

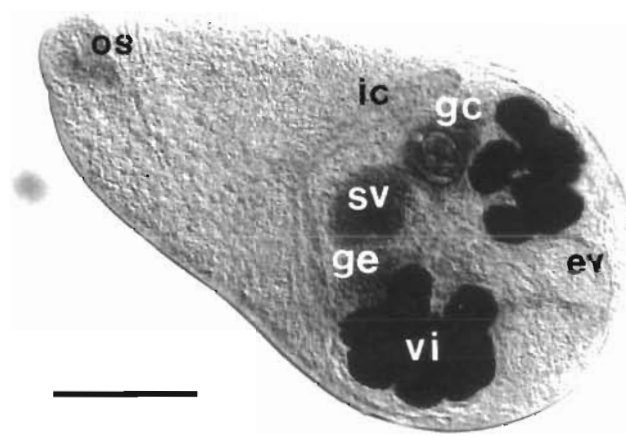

Fig. 1. Ventral view of a *Microphallus szidati* metacercaria from the hepatopancreas of *Chasmagnathus granulata*. ev: excretory vesicle; ic: intestinal cecae; gc: genital complex; ge: germarium; os: oral sucker; sv: seminal vesicle; vi: vitellarium. Bar = 0.15 mm

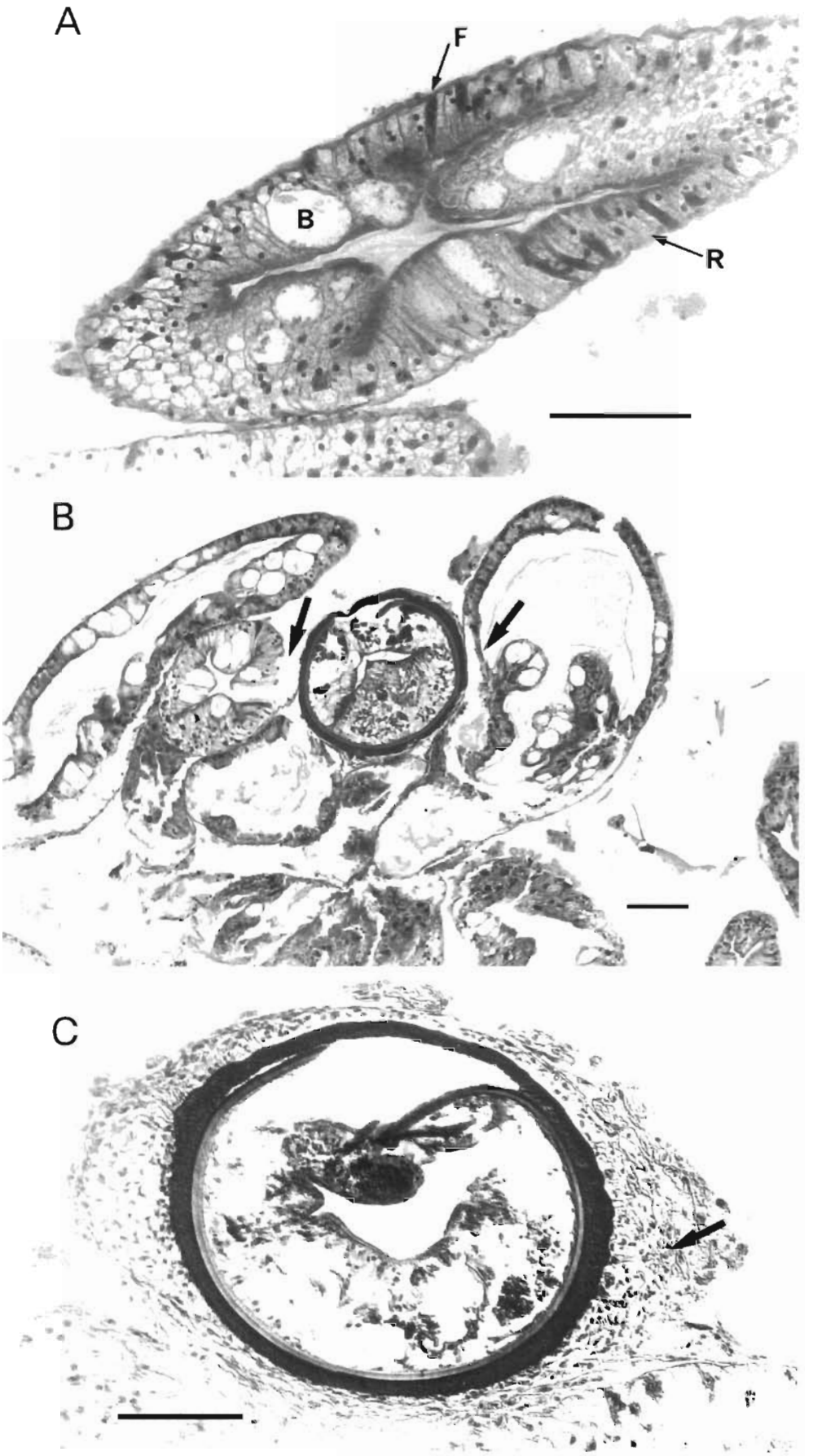

Fig. 2. (A) Non-parasitized hepatopancreas of *Chasmagnathus granulata* showing the cellular types F, B and R. (B) Epithelial delamination (arrows) in hepatopancreatic tubules of *C. granulata* in the presence of *Microphallus szidati* cysts. (C) Haemocyte infiltration (arrow) surrounding an *M. szidati* cyst in a hepatopancreatic tubule of *C. granulata*. Bars = 0.1 mm

was also similar to that of the glycogen determination method (5.3%). The biological variability observed for glucose and glycogen content of the hepatopancreas was estimated as 38.0 and 52.6%, respectively. The mean (±1 SE) content of glucose, glycogen, and lipids in the parasites was 0.7 ± 0.08; 12.0 ± 0.4; and 167.0 ± 24.0 µg cyst^{-1}, respectively.

According to the description of Johnston (1980), cellular types F (Fibrillenzellen), B (Blasenzellen) and R (Resorptionzellen), were recognized in the hepatopancreatic tubules of *Chasmagnathus granulata* (Fig. 2A). The parasite produced histopathology in hepatopancreatic tubules when the index of parasitism exceeded 6 parasites per tissue section (Fig. 2B). In this case, cellular debris was frequently observed in the lumen. Sometimes, a host response to the parasite was evident with haemocyte infiltration around the cyst (Fig. 2C).

Discussion. Several authors have reported the presence of parasites of the Microphallidae family in crabs and shrimp (Overstreet et al. 1992, Bush et al. 1993). Of these crustaceans, the grapsid crab *Cyrtograpsus angulatus* was considered by Martorelli (1989) as the principal second intermediate host for *Microphallus szidati*, whereas *Chasmagnathus granulata* could be an alternative intermediate host. Since the parasite larvae actively seek the host, it can be hypothesized that the crab distribution could be the main factor influencing the infection process. *C. angulatus* is an infralittoral crab, and hence could be more exposed to cercariae than *C. granulata* which is frequently found emersed (Boschi 1964). However, the scarce quantitative data on *C. angulatus* and *C. granulata* are not sufficient to support this idea.

The total lipids determined in the parasite cysts included triglycerides, phospholipids, glycolipids, etc. (i.e. not only storage lipids, but other kinds of lipids, including structural ones). However, as total lipid content was almost 14 times higher than glycogen content in *Microphallus szidati*, they were probably the main storage compounds. In fact, Zdárská (1964) reported that, over a period of 6 wk, the amount of glycogen in the tissues of *Echinostoma revolutum* metacercaria decreased while lipids increased, suggesting a lipid storage.

Considering structural damage caused by parasite infection, Martorelli & Schuldt (1990) described lesions in the hepatopancreas of *Cyrtograpsus angulatus* infected with *Microphallus szidati*, which are similar to those of the present study. These authors postulated that cyst capsulae had an haemocytic origin, and were derived from a host reaction. In accordance with these authors, we found in *Chasmagnathus granulata* a similar structural form of metacercariae capsulae, consisting of a wide external wall and a thin internal one (Fig. 2C). According to Martorelli & Schuldt (1990), hyaline, semigranular and granular haemocytes are responsible for capsulae formation when antigenic substances are bigger than these cells. The first haemocyte layers which surround the parasite suffer karyolysis, giving the hyaline characteristic to the inner capsulae wall (Fig. 2C). Autolysis of hepatopancreatic tubules, as observed in the present study, was also described by Martorelli & Schuldt (1990) in parasitized hepatopancreatic tissue of *C. angulatus*. Further, this process was sometimes followed by haemocyte infiltration (Fig. 2C). At this point, it is important to note that haemocytes constitute an important glycogen store in *C. granulata* (Nery & Santos 1993). Thus, haemocyte infiltration promoted by *M. szidati* metacercariae might explain the higher glycogen levels found in the hepatopancreas of infected crabs.

To summarize, there is evidence that *Microphallus szidati* metacercariae affect the glycogen levels in the hepatopancreas of *Chasmagnathus granulata*. Thus, the infection status of the crabs to be employed in future carbohydrate metabolism studies on this organ cannot be disregarded. Moreover, our results indicate that *M. szidati* metacercariae caused histopathological lesions in the hepatopancreas of *C. granulata*. Considering that these lesions were similar to those induced by organophosphate pesticide exposure in the prawn *Penaeus monodon* (Baticados & Tendencia 1991) or by copper exposure in the shrimp *Metapenaeus dobsoni* (Manisseri & Menon 1995), it is possible that the presence of parasites in the hepatopancreas of aquatic organisms could mask the influence of other stressors.

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