Glucose metabolism in the hepatopancreas of the crab *Neohelice granulata* maintained on carbohydrate-rich or high-protein diets: Anoxia and recovery

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**Abstract**

The effects of 1-h anoxia and 3-h recovery periods were assessed on hepatopancreatic glucose metabolism in the crab *Neohelice granulata* fed on either a carbohydrate-rich (HC) or high-protein (HP) diet. Anoxia increased pyruvate kinase (PK) and hexokinase (HK) activities in HC-fed animals, coinciding with a decrease in glycogen levels. No significant changes occurred in glucose uptake and glycogen synthesis in either dietary group. In HP-fed crabs, HK activity decreased and PK activity was 40% lower than that found in the HC-fed group after anoxia. Also, anoxia decreased 14C2O2 production, ATP and glycogen levels in hepatopancreas in both groups. During recovery, hemolymph glucose levels decreased in both groups but glucose uptake remained constant. In the HP group, the increase in glycogen synthesis represents an important fate of glucose during recovery from anoxia. In HC-fed crabs, the hepatopancreas glycolytic pathway is involved in the post-anoxia fate of glucose.

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**1. Introduction**

*Neohelice granulata* is a semi terrestrial crab that lives in the midlittoral and supralittoral zones of estuaries along the southern Brazilian coast, where it is an opportunistic feeder (D’Incao et al., 1990). In its habitat, *N. granulata* is exposed to environmental challenges such as changes in temperature, photoperiodicity, environmental oxygen partial pressure, food availability and composition, and salinity. These parameters may vary seasonally, and may change metabolic patterns (Chittó et al., 2009; Kucharski and Da Silva, 1991; Oliveira et al., 2004; Schein et al., 2004, 2005).

Glucose concentration in crustaceans hemolymph rises in response to a number of stress agents such as handling (Bergmann et al., 2001; Paterson and Spanoghe, 1997), emersion (Bernasconi and Uglow, 2008; Speed et al., 2001) salinity (Bianchini et al., 2008; Da Silva and Kucharski, 1992), and pollutants (Reddy et al., 1996). As in most other anoxia-tolerant crustaceans (Henry et al., 1994; Hervant et al., 1997; Hill et al., 1991; Paschke et al., 2010; Santos and Keller, 1993; Spicer et al., 2002), anaerobic metabolism in *Neohelice granulata* relies on the coupled fermentation of glycogen, with an accumulation of glucose and lactate as end products (Maciel et al., 2008; Oliveira et al., 2001; 2004). However, upon return to oxygenated water, *N. granulata* clears lactate via hepatopancreatic and muscular gluconeogenesis (Maciel et al., 2008; Oliveira et al., 2004).

Previous studies with *N. granulata* showed that the carbohydrate or protein contents in the diets administered to crabs induce different metabolic adjustments during anoxia and post-anoxia recovery period (Marqueze et al., 2006; Oliveira et al., 2001, 2004).

After 1-h anoxia, pyruvate kinase (PK) activity increased in jaw muscle from *N. granulata* fed on a high-protein diet (HP); in contrast, a high carbohydrate diet (HC) induced a reduction in this enzyme’s activity (Marqueze et al., 2006). During recovery (3 h), PK activity in jaw muscle decreased in crabs fed on an HP diet, and remained low in crabs receiving an HC diet (Marqueze et al., 2006). Anoxia increased the 14C-lactate incorporation into 14C-glycogen in the hepatopancreas of crabs fed on an HP diet or an HC diet (Oliveira et al., 2004). In post-anoxia recovery, in HP maintained crabs, the fate of L-lactate was hepatopancreas gluconeogenesis. In contrast to HP results, in crabs fed on an HC diet the hepatopancreas gluconeogenesis was not involved in L-lactate fate (Oliveira et al., 2004).

The maintenance of large stores of glycogen in all tissues under aerobic conditions is an adaptation feature that allows surviving in hypoxia or anoxia environments, and has been identified in different crustacean species (Childress and Seidel, 1998; Hervant et al., 1995; Storey and Storey, 2007). Short periods of anoxia and hypoxia are part of the normal biological cycle of *N. granulata*, and glycogen is an important fermentable substrate for this animal (Maciel et al., 2008; Oliveira et al., 2004). In this scenario, the following questions remain to be addressed: Is there a difference in the hepatopancreatic glycolytic flux in response to short periods of anoxia and post-anoxia...
recovery in crabs having different concentrations of fermentable substrate in their tissues? How is glucose metabolized in the hepatopancreas during the post-anoxia recovery period in crabs with different initial concentrations of glycogen? To answer these questions, we evaluated the effects of short periods of anoxia and recovery from anoxia on the glycolytic flux, glucose oxidation and uptake and glycogen synthesis in the hepatopancreas of crabs with high (HC fed crabs) and low (HP fed crabs) glycogen concentrations.

2. Materials and methods

2.1. Animals

Male Neohelice granulata in stage C of the intermolt cycle, according to the morphological criteria described by Drach and Tchernigovtzeff (1967), were collected in Lagoa Tramandaí, (S 29° 58′ 25.5″ W 50° 08′ 18.5″). The experimental protocol followed the Brazilian Environmental Law. Every effort was made to minimize the number of animals used.

2.2. Experimental procedure

Animals weighing 16 ± 2 g were placed in aquaria at a salinity of 20‰, at 25 °C, and a natural light/dark cycle.

Crabs were divided into two groups, one was fed on a high-protein/low-carbohydrate diet (HP, beef), while the other was fed on a carbohydrate-rich diet (HC, boiled rice) according to Kucharski and Da Silva (1991). Both groups were fed daily ad libitum (about 50 g) in the late afternoon for two weeks before the experiments. There was no variation in the body mass (16.5 ± 0.5 g) of crabs in either group during the experimental period.

For the anoxia study, pools of 14 animals receiving either the HC or the HP diet were placed in glass aquaria (30 × 25 × 30 cm; 20 L) at a salinity of 20‰ and a temperature of 25 °C. The water in which the crabs were immersed was bubbled with N2 for 40 min until oxygen concentration reached zero (monitored with an ISO2/Oxel-1, World International). Following incubation, the hepatopancreas was rinsed with normoxic water. After 3 h in normoxic water, the recovered animals were kept under anoxic conditions for 1 h, after which they were transferred to vials containing 5 mL scintillation liquid. The tissue samples were used in the experiments.

For measurement of glycogen synthesis, hepatopancreas fractions (50 ± 2 mg) from the different experimental groups were incubated at 25 °C for 60 min in 500 μL incubation buffer in 0.2 μCi [U-14C] glucose (230 mCi mmol−1, Amersham International) plus 10 mM glucose. For 14C-glycogen determination, the hepatopancreas fractions were removed, rinsed in cold incubation buffer, blotted with filter paper, immediately transferred to KOH (0.5 N), and boiled for 60 min; proteins were precipitated in 30% trichloroacetic acid and 1 N HCl (2:1, v/v). After centrifugation at 600 g for 10 min, the supernatant (30 μL) was applied on Whatman 3MM strips. Glycogen was precipitated with 66% ethanol at final concentration (Thomas et al., 1968). As a blank, the strip, without the tissue sample, was subjected to the same procedure. The wet strips were transferred to vials containing 5 mL scintillation liquid. Glycogen synthesis rates are given as nmol of 14C glucose incorporated into glycogen per mg−1 of tissue min−1.

2.5. 14CO2 production

Glucose oxidation was determined according to Torres et al. (2001). Hepatopancreas samples (50 ± 2 mg) were incubated in flasks sealed with rubber caps at 25 °C in 1 mL of incubation buffer in 0.2 μCi [U-14C] glucose (230 mCi mmol−1, Amersham International) plus 10 mM glucose for 60 min, according to Marqueze et al. (2006). Saturating glucose concentrations were used for CO2 production.

In these flasks, small glass wells situated above the level of the incubation medium contained small strips of Whatman 3MM paper. Next, 1 M Hyamine® hydroxide (ammonium, benzylidemethyl (2-24-(1,3,3-tetramethylbutyl) tollyoxy) ethyl)-chloride solution in methanol was injected (0.2 mL) in the central wells to trap 14CO2. Incubation was stopped by adding 0.2 mL of 50% TCA through the rubber cap. The flasks were shaken for further 60 min at 25 °C to trap 14CO2. The contents of the center well were transferred to vials with scintillation liquid and radioactivity was counted using an LKB counter. Values of 14CO2 production were expressed as mmol of 14C glucose incorporated into CO2 per mg−1 of tissue min−1.

2.6. Pyruvate kinase activity

Pyruvate kinase (PK) (EC 2.7.1.40) activity in the hepatopancreas was determined according to Feska et al. (2003). Samples of hepatopancreas (500 mg) from the different experimental groups were homogenized (1:5 w/v) in ice-cold buffer (in mM) 0.32 sucrose, 1 mM EGTA (ethylene glycol tetra acetic acid), 10 Tris–HCl, pH 7.4, and 0.1 PMSF with a Teflon pestle homogenizer. The homogenate was centrifuged at 10,000 g for 15 min at 4 °C. The pellet was discarded, and the mitochondria-free supernatant was used for the PK enzyme assays and determinations of protein concentration. Pyruvate kinase activity was assayed in incubation medium containing (in mM) 10 M Tris–HCl, 10 MgCl2, 0.16 NADH, 75 KCl, 5.0 ADP, 1.0 U of L-lactate dehydrogenase, pH 7.5, 0.1% (v/v) Triton X-100, and 10 μL of the mitochondria-free supernatant in a final volume of 0.5 mL. Unless stated otherwise, the reaction was using 1.0 mM phosphoenolpyruvate at final concentration. All assays were performed in duplicate at 25 °C. Results were expressed as μmol of transformed substrate per mg of protein−1 per min−1.

2.7. Hexokinase activity

Samples of hepatopancreas from the different experimental groups were removed and immediately homogenized (1:3, w/v) (Potter-Elvehjem) in ice-cold buffer (in mmol/L): 50 Tris–HCl 5
MgCl₂, 1 EDTA, 20 mercaptoethanol, pH 8.2, and 0.1 mM of PMSF. Next, the homogenate was centrifuged at 18,000 g for 20 min at 5 °C. Aliquots of the supernatant were used to determine enzyme activity.

Hexokinase (HK) [EC 2.7.1.1] activity was assayed essentially as described by Brito et al. (2001). In addition to 0.1 mL of supernatant, the HK assay medium contained (in mmol/L): 75 Tris–HCl, 7.5 MgCl₂, 0.8 EDTA, 1.5 KCl, 4.0 mercaptoethanol, 0.4 nicotinamide-adenine dinucleotide phosphate (NADP)⁺, 2.5 ATP, 10 creatine-phosphate, 1 glucose, and creatine phosphokinase (100 g, 1.8 units), and glucose-6-phosphate dehydrogenase (10 g, 1.4 units). Controls from which the glucose was omitted were run concurrently. The reaction was started with 0.4 mmol/L of NADP⁺. All assays were performed at 25 °C. The results were expressed as μmol per min⁻¹ per mg⁻¹ protein.

### 2.8. Chemical analyses

Protein was determined by the Bradford method (1976), using bovine albumin as standard. Hepatopancreas glycogen was extracted according to Van Handel (1965), determined as glucose after acid hydrolysis, and expressed as g of glycogen per 100 g of tissue. The glucose concentration was determined by the enzymatic oxidase method (Labtest kit) and expressed as mmol L⁻¹ of glucose. ATP was determined in hepatopancreas (1 g) from the different experimental groups. Samples of hepatopancreas were homogenized (1:5 w/v) in ice-cold 6% TCA with an Omni Mixer homogenizer. The homogenate was centrifuged at 10,000 g for 3 min at 4 °C. The pellet was discarded, and the supernatant was used to determine ATP with a Sigma Diagnosis Kit. ATP values are expressed as μmol ATP mg⁻¹ of tissue.

### 2.9. Statistical analysis

Results were expressed as means±SEM. All the data passed the test for normality and variance. Data were analyzed by two-way (for HC and HC groups during normoxia, anoxia or recovery treatment) analysis of variation (ANOVA) followed by Student-Newman-Keuls (SNK) comparison test. P<0.05 was taken as the criterion of significance. All tests were performed with SPSS for Windows.

### 3. Results

In all the results there is a statistically significant interaction between diets and normoxia, anoxia or recovery treatment.

In the normoxic control group, hemolymph glucose level in HC-fed animals was 2.8 times higher (P<0.05) than in HP-fed crabs (Table 1). After 1-h anoxia, hemolymph glucose concentrations in HP and HC-fed crabs increased, and reached levels 7.5 (P<0.05) and 4 (P<0.05) times higher than control group levels, respectively. In anoxia, the glucose concentration in the hemolymph of crabs receiving the HC diet was higher (P<0.05) than in the HP group. In both dietary groups, hemolymph glucose levels decreased (P<0.05) after 3 h of recovery as compared to anoxia group. However, in HC-fed crabs remained higher (P<0.05) than those of the control group (Table 1).

![Fig. 1. Glucose uptake during anoxia and subsequent recovery in hepatopancreas of crabs fed a high-protein (HP) or a carbohydrate-rich (HC) diet. Values represent mean±SEM, n=6–10 animals in each experiment.](image_url)

The ATP concentration in the hepatopancreas of crabs receiving the HC diet was higher (P<0.05) than in the HP group. After 1-h anoxia, the ATP concentration in the hepatopancreas from both dietary groups decreased (P<0.05) as compared to control animals. In the HC group, the ATP concentration was lower (P<0.05) than in HP-fed animals. During the recovery from anoxia, the ATP content in the hepatopancreas from both dietary groups increased (P<0.05) when compared to the value observed in anoxia. In the HP-fed crabs, the ATP content in the hepatopancreas reached a concentration similar to that found in the control group after 3 h in recovery. However, in HC-fed animals the ATP value after the recovery period corresponded to 39% of the control group concentration (Table 1).

The glycogen concentration in the hepatopancreas of crabs receiving the HC diet was higher (P<0.05) than in the HP group. The hepatopancreas glycogen content decreased (P<0.05) after 1 h in anoxia only in HC-fed group. During the recovery period the glycogen level decreases (P<0.05) in HP group, and remained similar to anoxia group in HC-fed crabs. However, the difference (P<0.05) between glycogen levels in the HP and HC groups observed in the control group was observed in crabs submitted to anoxia and recovery (Table 1).

Differences in 2DG uptake were not found between crabs fed the HP diet and those maintained on the HC diet (Fig. 1). No differences were observed in glucose uptake between the control, anoxia, and recovery groups, for animals fed on either diet (Fig. 1).

In control crabs, no significant difference was observed in glycogen synthesis in the hepatopancreas between animals fed on the HC and HP diets (Fig. 2). Anoxia for 1 h did not significantly affect glycogen synthesis in the hepatopancreas in either experimental group (HP and HC). In contrast, during recovery, the capacity for glycogen synthesis in the

### Table 1

<table>
<thead>
<tr>
<th>Groups</th>
<th>Glucose (mmol L⁻¹)</th>
<th>ATP (μmol g of tissue⁻¹)</th>
<th>Glycogen (g%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HP</td>
<td>HC</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.4 ± 0.18</td>
<td>1.1 ± 0.07</td>
<td>1.44 ± 0.06</td>
</tr>
<tr>
<td>Anoxia</td>
<td>3.7 ± 0.53</td>
<td>4.6 ± 0.23</td>
<td>0.93 ± 0.03</td>
</tr>
<tr>
<td>Recovery</td>
<td>1.0 ± 0.13</td>
<td>1.9 ± 0.32</td>
<td>1.47 ± 0.07</td>
</tr>
</tbody>
</table>

Values represent mean±SEM, (n=6–10 animals in each experiment).

a Mean values are different from the control group (P<0.05).
b Mean values are different from the anoxia group (P<0.05).

* Mean values are different from the HP group (P<0.05).
hepatopancreas of the HP group increased by about 8 ($P<0.05$) and 5 times ($P<0.05$), when compared to those of the control and anoxia groups, respectively. This significantly increase was also compared to that observed in the recovery group fed on the HC diet (Fig. 2).

In the control group, the $^{14}$C-glucose oxidation in the hepatopancreas of crabs receiving the HC diet was lower ($P<0.05$) than in the HP group (Fig. 3). After 1 h in anoxia, $^{14}$C-glucose oxidation in the hepatopancreas of the HP group was about 64% ($P<0.05$) lower, than in the hepatopancreas of control HP-fed crabs. After 3 h in recovery, no significant difference in $^{14}$C-glucose oxidation was observed in the HP group, as compared to anoxia. In HC-fed crabs, anoxia for 1 h or recovery for 3 h did not significantly affect the $^{14}$C-glucose oxidation in the hepatopancreas. However, in HC-fed crabs the $^{14}$C-glucose oxidation in the hepatopancreas from crabs submitted to anoxia was significantly higher, than in the hepatopancreas of HP-fed animals (Fig. 3).

In the control HC group, PK activity in hepatopancreas was 81% higher ($P<0.05$) than in the HP group. After 1 h exposed to anoxia, increased ($P<0.05$) PK activity was observed in both diet groups (Fig. 4A). However, in the HC group, PK activity was twice ($P<0.05$) as high as in the HP-fed crabs. PK activity decreased ($P<0.05$) in HP animals as they were exposed to recovery. Still, PK activity was similar in the anoxia and post-anoxia period in HC animals. However, in HC-fed animals was 3.5 times higher ($P<0.05$) than in HP-fed crabs (Fig. 4A).

Fig. 4B shows that in the control group, HK activity was significantly higher ($P<0.05$) in HC-fed animals than in the HP-fed group. After 1 h in anoxia, enzyme activity increased ($P<0.05$) in the HC group. However, in HP-fed crabs, HK activity decreased about 66% ($P<0.05$) after 1 h in anoxia. In the recovery period, in the HC-fed crabs, HK activity decreased 40% ($P<0.05$) and 53% ($P<0.05$) as compared to the control and anoxia groups, respectively. Conversely, HK activity in the hepatopancreas from HP-fed crabs increased about

![Fig. 2. Glycogen synthesis during anoxia and subsequent recovery in hepatopancreas of crabs fed a high-protein (HP) or a carbohydrate-rich (HC) diet. Values represent mean±SEM, n=6–10 animals in each experiment. * Mean values are significantly different from the control group (P<0.05). † Mean values are significantly different from the anoxia group (P<0.05). ‡ Mean values are significantly different from the HP group (P<0.05).](image1)

![Fig. 3. Effects of anoxia and recovery on $^{14}$CO$_2$ formation in hepatopancreas of crabs fed a high-protein (HP) or a carbohydrate-rich (HC) diet. Values represent mean±SEM, n=6–10 animals in each experiment. * Mean values are significantly different from the control group (P<0.05). † Mean values are significantly different from the anoxia group (P<0.05). ‡ Mean values are significantly different from the HP group (P<0.05).](image2)

![Fig. 4. Activities of pyruvate kinase A and hexokinase B during anoxia and subsequent recovery in hepatopancreas of crabs fed a high-protein (HP) or a carbohydrate-rich (HC) diet. Values represent mean±SEM, n=10 animals in each experiment. * Mean values are significantly different from the control group (P<0.05). † Mean values are significantly different from the anoxia group (P<0.05). ‡ Mean values are significantly different from the HP group (P<0.05).](image3)
threefold (P<0.05) during the recovery period, reaching levels close to those of the control group (Fig. 4B).

4. Discussion

The present data show that administration of an HC diet results in a marked increase in hepatopancreatic glycolytic flux in normoxic N. granulata, as evidenced by the higher activities of the glycolytic enzymes HK and PK. These findings show that in N. granulata hepatopancreas the HK and PK activities were under the nutritional regulation. This result agrees with the high PK activity found in jaw muscle of HC-fed N. granulata, when compared to that observed in the group fed on the HP diet (Marqueze et al., 2006). Also, in fish fed on high starch diet, the hepatic PK activity increased (Enes et al., 2006; Tan et al., 2009), indicating carbohydrate control over the enzyme activity. Contrasting with our results, the hepatic HK activity in fishes was not affected by the absence or the presence of starch in the diet (Panserat et al., 2000; Tan et al., 2009). On the other hand, no effect of diet composition on hepatopancreatic glucose uptake, and glycogen synthesis was observed in normoxia crabs. In contrast, in the HC-fed group the 14CO2 production from 14C-glucose was three times lower than in the HP group. This result may be explained by the higher ATP and glycogen levels found in hepatopancreas from HC-fed crabs.

For a variety of animals, including N. granulata, access to environmental oxygen is intermittent. This semi terrestrial crab undergoes daily episodes of hypoxia during its forays on land. In winter, this crab faces hypoxia or anoxia when remaining in its holes for long periods with a low oxygen supply (Turcato, 1990).

Like other crustaceans, there was a marked increase in the concentration of hemolymph glucose after 1 h in anoxia in HC- and HP-fed crabs, suggesting that glucose mobilization in the tissues exceeds its utilization in the glycolytic pathway, causing an accumulation of glucose in the hemolymph (Anderson et al., 1994; Hervant et al., 1997; Van Aardt, 1988; Zou et al., 1996). Furthermore, the concentration of hemolymph lactate increases 5–14 times in N. granulata fed an HC or HP diet and subjected to hypoxic or anoxic stress for different periods of time (Maciel et al., 2008; Oliveira et al., 2004).

Vertebrates and invertebrates show a biphasic response to a reduction in oxygen levels in the environment (Lutz and Storey, 1997; Storey and Storey, 2007). The first phase leads to the activation of the glycolytic pathway to maintain or increase ATP production, since the oxygen-dependent production pathways may be significantly reduced. The second phase would lead to the reduction in metabolic rate, which affords the gradual use of endogenous reserves and survival for prolonged periods of severe hypoxia or anoxia, as observed in turtles and mollusks (terrestrial, bivalve, and cephalopod) (Churchill and Storey, 1989; Guppy et al., 1994; Seibell and Childress, 2000).

The results of this study demonstrate that the hepatopancreas from HC crabs were in a transition state, from normoxia to hypoxia, characterized by the increase in PK and HK activities, which led to an increase in glycolytic flux. In this group, the activation of the hepatopancreatic glycolytic flux during anoxia coincided with the decline in glycogen levels in this organ. In contrast, 1-h anoxia decreased PK activity in jaw muscle of N. granulata fed on an HC diet (Marqueze et al., 2006). These results agree with Chang's (2005) observation that in lobster submitted to hypoxia stress the animal's tissues do not respond proportionately to a given stress. This difference may be explained by a late activation in the glycolytic flux in hepatopancreas as compared to muscles (Marqueze et al., 2006). In muscles of crabs fed on HC diet, the decrease in PK after 1 h in anoxia was followed by an increase in 14CO2 production from 14C-glucose, suggesting that glucose is diverted from the glycolytic pathway to the pentose phosphate pathway, which can operate to oxidize glucose completely to CO2 and water (Marqueze et al., 2006). However, in hepatopancreas from HC-fed crabs the 14CO2 production from 14C-glucose remained unchanged during the anoxia period, suggesting a bypass of the glucose to the glycolytic flux. These results agree with the marked glycogen mobilization found in hepatopancreas of crabs fed on an HC diet.

In contrast, no significant changes were observed in glucose uptake and glycogen synthesis in the hepatopancreas of crabs fed on the HC diet measured after 1 h in anoxia. These results may be explained by the high glycogen mobilization as a consequence of the high glycogen content found in HC-fed hepatopancreas animals.

Unlike the HC-fed crabs, the HP-diet group was already entering the second stage of response to reduction in O2 levels in the environment. This statement is based on the observation that after 1 h in anoxia, in the crabs fed on the HP diet, HK activity decreased markedly and PK activity in hepatopancreas was 40% lower than that found in the HC-fed group. Similarly, in hepatopancreas from HP-fed crabs, the 14CO2 production from 14C-glucose decreased with the reduction in oxygen levels.

In the hepatopancreas from HP-fed group the HK activity decreased during anoxia, suggesting an increase in the percentage of dissociation HK enzyme from active complex as observed in Otala lactea (Plaxton and Storey, 1986). This mechanism appears, therefore, to be a simple and fast way to change the flux through glycolysis in a readily reversible fashion. In anoxia, the increase in glucose concentration in the hemolymph of the HP-fed crabs was 5 times higher than that found in HC-fed animals, despite the low glycogen content and mobilization in hepatopancreas of this dietary (HP) group, suggesting a marked reduction in the utilization of glucose by glycolytic flux during anoxia stress. These results suggest that glycolysis in RC-fed crabs increased with anoxia, a response possibly associated with the diet. In contrast, animals fed on the HP the HK activity decreased, suggesting that in those crabs the glycolysis pathway was not affected by hypoxic conditions.

However, true anoxia did not yet occur in HP-fed crabs, because the rates of glucose uptake and glycogen synthesis are similar to those observed in normoxic animals. Oliveira et al. (2001) observed a decrease in glucose uptake and glycogen synthesis only after 2 h in anoxia.

After 1 h in anoxia, the concentrations of ATP in the hepatopancreas of crabs fed on the HP diet decreased about 1.5 times, while in the animals receiving the HC diet the reduction was of about 3 times. On the other hand, the preferential use of phosphagens stores in order to maintain a high ATP concentration and decrease glycolysis flux in the hepatopancreas during the first hour of anoxia, as observed in other crustaceans and mollusks (Gade, 1983, 1984; Hervant et al., 1995; 1997; Isani et al., 1989), may explain the maintenance of high ATP and glycogen contents in HP-fed crabs. On the other hand, the high hemolymph glucose levels could inhibit the mobilization of fuel reserves in the hepatopancreas; or in HP-fed crabs the pool of free amino acids product of the catabolism of the alimentary and/or muscular proteins during the first hour of anoxia may be used as an energy source as demonstrated in Penaeus setiferus juveniles submitted to prolonged hypoxia (Rosas et al., 1999). Ultimately there could be a decrease in the total adenine nucleotide pool without a change in the energy state ([ATP]/[ADP]/Pi) ratio. These possibilities that need to be investigated.

The post-anoxia recovery process has great functional importance, since it is during recovery that energy reserves are restored and the accumulated end products are removed from the organism.

During the 3 h of post-anoxia recovery, hemolymph glucose levels decreased in both dietary groups, though without reaching the levels seen in the normoxia group. However, the post-anoxia recovery did not affect glucose uptake in the hepatopancreas in either diet group. Thus, in the HP-fed animals, along with the return of HK activity to levels similar to those of the control group, the increase in the capacity of glycogen synthesis represents an important fate of glucose during recovery from anoxia. Taking into account that PK activity in the hepatopancreas of the recovery HP-group was similar that found in normoxia group, another alternative pathway for glucose fate is the aerobic glycolysis. This hypothesis is corroborated by increase in ATP concentration in HP-fed crabs hepatopancreas after the 3-h recovery period. Also, in N. granulata
fed on an HP diet, the jaw muscle is an important organ in reprocessing glucose in the 3-h post-anoxia recovery period, increasing glucose oxidation and glycolytic flux (Marqueze et al., 2006).

In *N. granulata* fed on the HP diet, the glycolytic pathway in the hepatopancreas is one pathway involved in the post-anoxia fate of glucose. During recovery, HK activity decreased in the group fed on the HP diet, although this activity level was similar to that observed in the HP group in recovery. However, in spite of the decline in HK activity, the contribution of the glycolytic flux to the utilization of glucose and recovery of the energy reserves cannot be neglected. In this diet group, the ATP concentration in the hepatopancreas increased compared to the anoxia group, suggesting an increase in energy reserves. The decrease in HK activity may have played a role in the maintenance of high hemolymph glucose levels and low glycogen concentration, although no change in glucose uptake was observed in HP-fed crabs.

In conclusion, it was possible to identify different metabolic strategies used by the hepatopancreas tissue from *N. granulata* subjected to 1 h of anoxia and 3 h of post-anoxia recovery, based on the glycogen content in the tissue. In the crabs fed on the HP diet, the significant reduction in HK and PK activities, when compared to those observed in the HC group after 1 h of anoxia, suggest that the low concentration of hepatopancreatic glycogen observed in the HP group may induce a decrease in glycolytic activity. In the HC group, which showed high levels of glycogen in the tissues, the glycolytic activity was markedly increased after 1 h anoxia.

In post-anoxia recovery, glycogen synthesis represents an important fate of glucose in the hepatopancreas of HP-fed crabs, and glycolytic flux in the HC-fed group.

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