RAPID COMMUNICATION

Lipids as Energy Source During Salinity Acclimation in the Euryhaline Crab *Chasmagnathus granulata* Dana, 1851 (Crustacea-Grapsidae)

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ABSTRACT Lipids seem to be the major energy store in crustaceans. Moreover, they are extremely important in maintaining structural and physiological integrity of cellular and sub cellular membranes. During salinity adaptation, energy-demanding mechanisms for hemolymph osmotic and ionic regulation are activated. Thus, the main goal of this work was to verify the possible involvement of lipids as an energy source in the osmotic adaptation process. The estuarine crab *Chasmagnathus granulata* was captured and acclimated to salt water at 20‰ salinity and $20 \pm 2°C$, for 30 days. After acclimation, crabs were divided into groups of ten and transferred to fresh water (0‰), salt water at 40‰ salinity, or maintained in salt water at 20‰ salinity (control group), without feeding. Before and seven days after the salinity change, wet weight and lipid concentration in gills, muscle, hepatopancreas, and hemolymph were determined according to the colorimetric assay of sulphophosphovanilin. Results show that hepatopancreas lipids were not mobilized during osmotic stress regulation. Gill and muscle lipids were significantly lower in crabs subjected to hypo-osmotic stress than those subjected to the hyper-osmotic stress or maintained at the control salinity. Our results point to the occurrence of lipid mobilization and involvement of these compounds in the osmotic acclimation process in *C. granulata*, but with differences between tissues and the osmotic shock (hypo or hyper) considered. *J. Exp. Zool. 295A:200–205, 2003.* © 2003 Wiley-Liss, Inc.

Stress induced by changes in environmental conditions requires homeostatic and enanteostatic regulations that induce behavioral and physiological alterations in aquatic animals. Furthermore, stressing conditions also require the animal's ability to provide enough fuel to tissues in order to cope with a higher energy demand. In light of this, identification of what energy source is used and which tissues are mobilizing and providing this source is an important issue to understand metabolic aspects of homeostatic and enanteostatic regulations during stress.

In crustaceans, there is a lot of data available in the literature about this subject. Unfortunately, the data are usually diverse and sometimes contradictory. For example, proteins could be the major energy store, especially in Macruran species (Barclay et al., '83; Dall and Smith, '87). However, *Penaeus esculentus* can use both protein and lipid for energy requirements during starvation and moulting (Barclay et al., '83). Also during starvation, hepatopancreatic glycogen seems to constitute the major source of energy in the crab *Chasmagnathus granulata* (Vinagre and

Da Silva, '92), while in *Carcinus maenas*, lipids are used five-fold more than carbohydrates (Heath and Barnes, '70). Apparently, as pointed out by some authors, hepatopancreatic lipids are used only in large amounts in specific processes such as ecdysis (O'Connor and Gilbert, '69; Ando et al., '77; Chandumpai et al., '91) and reproduction (Armitage et al., '72; Clarke, '79; Jeckel et al., '91).

As far as osmotic stress is concerned, it has been demonstrated that euryhaline crabs, such as *C. maenas* and *Callinectes sapidus*, show increased blood protein content associated with decreased blood osmolality. This process might be related to the storage of amino acids leaking out of the cells during the volume control process (Péqueux et al., '79). Total proteins in the rotifer *Brachionus plicatilis* decrease with simultaneous increase in the free amino acid content due to an increase in

salinity from 0.5‰ to 28‰ (Frolov et al., '91). Similarly, postlarvae of the shrimp *Farfantepenaeus paulensis* showed lower protein concentration values at higher salinities (Lemos et al., 2001). In addition, it also seems that a straight relationship between environmental salinity and hemolymph glycemia in crustaceans occurs (Santos and Nery, '87; Spaargaren and Haefner, '87; Da Silva and Kucharski, '92). Even though glycogen metabolism is linked with osmotic adaptation, it does not seem to be the only source of energy in *C. granulata* during osmoregulation (Da Silva and Kucharski, '92; Nery and Santos, '93). The effect of external salinity on the lipid class composition and synthesis in crabs is well documented (Chapelle et al., '82; Chapelle and Zwingelstein, '84; Zwingelstein et al., '98). However, few reports verified whether lipid mobilization occurred during osmotic adaptation. To our knowledge, only Frolov et al. ('91) and Lemos et al. (2001) determined some degree of lipid mobilization on *B. plicatilis* and *F. paulensis*, respectively, during osmotic adaptation. In the present paper, lipid mobilization over time during hypo- or hyper-osmotic stress is described.

MATERIALS AND METHODS

C. granulata were captured in salt marshes near Rio Grande city (Southern Brazil) in winter (August) and summer (February). In the laboratory, crabs were acclimated to salt water at 20‰ salinity and $20 \pm 2°C$, for 30 days. During this period, they were fed ground beef three times per week. Only adult males (7.29 ± 2.86 g) in stage C or early D of the intermolt cycle were used. After acclimation, different groups of crabs were abruptly transferred to aquaria containing water at different salinities (0‰, 20‰, and 40‰) and kept without food. At the beginning of the experiments (time 0) and seven days after the salinity change, hemolymph was collected by puncture of the blood sinus at the base of the fourth or fifth pair of pereiopods. After hemolymph collection, crabs were crioanesthetized and had their carapace removed. Gills, muscle, and hepatopancreas were then dissected. Tissues were immediately frozen at $-20°C$ for a period no longer than 72 hr until lipid measurements. Lipid concentration in hemolymph and tissues was determined according to the colorimetric method of sulphophosphovanilin using a reagent kit (Lípides Totais® Doles S.A., Belo Horizonte, MG, Brazil). Samples were homogenized in a chloroform-methanol (2:1, v/v) mixture. Fat acids were then released by hydrolysis and reacted with vanilin in the presence of sulfuric acid.

An extra group of crabs was used to determine salinity effect on water content in gills and muscle. These crabs were collected and acclimated in the summer, as previously described.

Possible differences in lipid concentration between summer and winter crabs were verified using the Student *t*-test. To verify possible significant effects of salinity on lipid concentration and water content, data were subjected to analysis of variance (ANOVA one way) followed by the SNK test. The level of significance adopted was 5‰ ($\alpha = 0.05$).

RESULTS

Seasonal differences in lipid concentration were observed in gills, muscle, and hepatopancreas. These tissues showed higher lipid concentration values in crabs caught and acclimated in winter than in summer. However, hemolymph lipid concentration was similar in both seasons (Fig. 1). Therefore, analysis of lipid changes after osmotic shock was performed separately for each season.

In summer, gills and hemolymph of crabs subjected to hypo-osmotic stress (salinity 0‰) showed a lower total lipid concentration than those maintained in the control salinity (20‰). However, lipid concentration in hepatopancreas and muscle was not significantly affected by the hypo-osmotic shock.

A marked increase in lipid concentration was observed in all tissues analyzed when crabs were subjected to the hyper-osmotic stress (40‰ salinity) in summer. This increase was not significant ($P > 0.05$) in the muscle only.

In winter, lipid concentration decreased in gills and muscle after the hypo-osmotic stress. Although circulating lipids decreased by 29‰, there were no significant changes in both circulating and hepatopancreatic lipid concentration even after one week of hypo-osmotic stress. After the hyper-osmotic stress, only gills showed a significantly different (higher) lipid concentration from that observed in gills of crabs maintained in the control salinity (20‰).

Before salinity transference, water content in muscle and gills of crabs acclimated to 20‰ salinity was 0.769 ± 0.006 g water/g^{-1} WW (mean ± 1 SE) and 0.725 ± 0.009 g water/g^{-1} WW, respectively. After the hypo-osmotic stress, a small
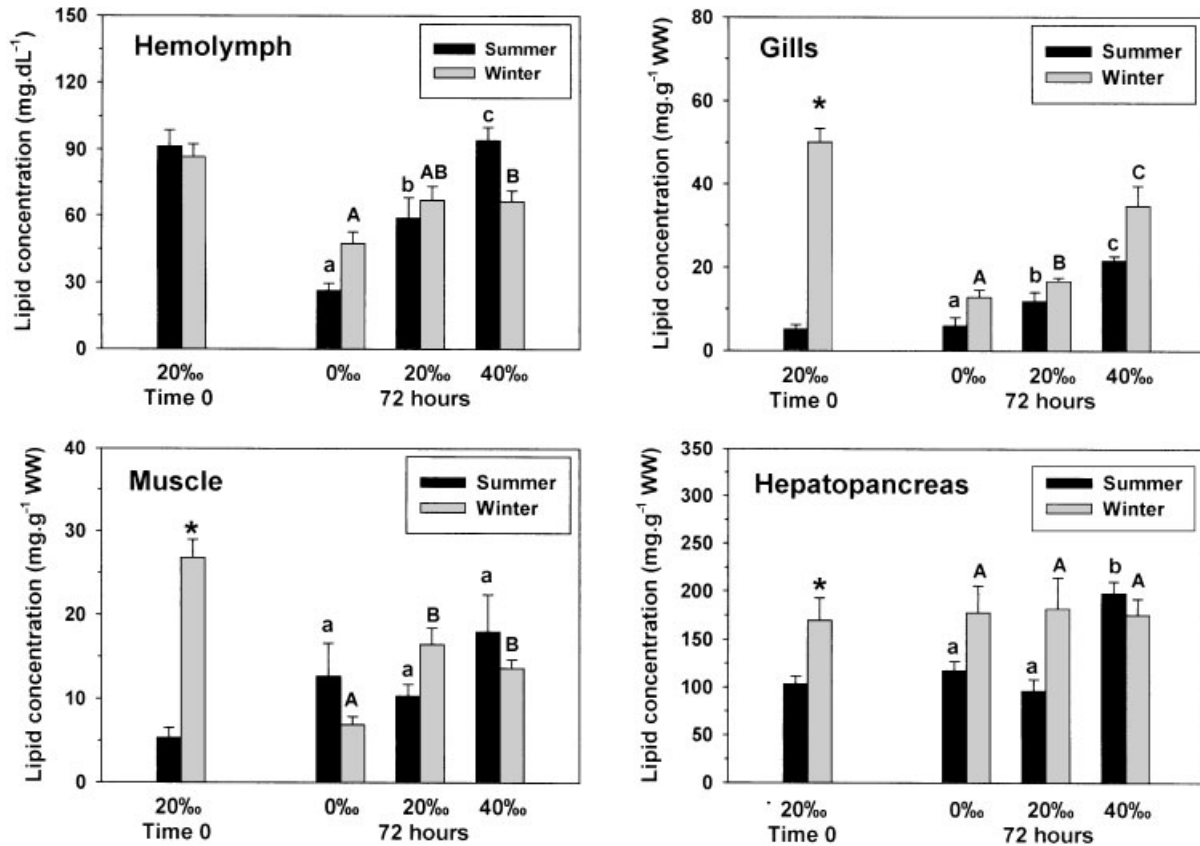
Fig. 1. Total lipid concentration in hemolymph, gills, muscle, and hepatopancreas of *Chasmagnathus granulata* before (time 0) and seven days after transfer to a new salinity (0‰, 20‰, or 40‰) in summer and winter. Data are mean values ± 1 standard error (n = 6–10 crabs). *indicates significantly (*P* < 0.05) different mean values between seasons. Different letters indicate significantly (*P* < 0.05) different mean values between salinities within the same tissue and season.

but significant increase (*P* < 0.05) in water content was observed in both muscle (0.800 ± 0.004 g water/g^{-1} WW) and gills (0.769 ± 0.007 g water/g^{-1} WW). After hyper-osmotic stress, a significant water loss was observed in both muscle (0.757 ± 0.004 g water/g^{-1} WW) and gills (0.606 ± 0.02 g water/g^{-1} WW).

DISCUSSION

Lipid content in several tissues (gills, muscle, and hepatopancreas) of *C. granulata* was significantly different in winter and summer, with values being lower in summer. Similar results were observed in the crayfish *Orconectes nais* (Armitage et al., '72), since hepatopancreas and muscle lipid content decreased in midsummer for both sexes. Tsai et al. ('84) demonstrated that lipid and cholesterol consumption exceed their production in gonad, hepatopancreas, and muscle of mature males of *Callinectes sapidus*. In the shrimp *Macrobrachiun borellii*, lipid content in

muscle, gills, and especially hepatopancreas, also changed over the year, with the highest values being found in winter (Gonzalez-Baro and Pollero, '88). All of these results are pointing to a metabolic strategy used by crustaceans to store energy compounds that will be useful during winter adversities.

Despite the seasonal difference in lipid content, our results demonstrated that salinity stress induced similar changes in tissue lipid content in both seasons. A decrease in lipid concentration was observed after the hypo-osmotic stress, while an increase was observed after hyper-osmotic stress. Although the effect of salinity on the concentration of different lipid classes and their variation in fatty acid composition are well reported in the literature (Chapelle et al., '82; Zwingelstein et al., '98), studies looking at the effect of salinity on total lipid concentration in crustacean tissues are still scarce. Frolov et al. ('91) described a decrease in lipid content in the rotifer *B. plicatilis* at salinities lower or higher

than 17‰. Lemos et al. (2001) also recently demonstrated salinity change-induced alteration in lipid content in early postlarvae *Farfantepenaeus paulensis*. These authors showed that lipid concentration in postlarvae VI–VII was lower at 5‰ salinity. However, in postlarvae VIII–XV, no changes were observed.

As reported for other crustaceans, hepatopancreas of *C. granulata* showed higher lipid concentration than other tissues. However, our results suggest that hepatopancreatic lipids are not involved as energy sources in the salinity adaptation in *C. granulata*. In fact, previous studies have demonstrated that hepatopancreatic lipids are involved in physiological processes in crustaceans such as molt (O'Connor and Gilbert, '69; Ando et al., '77; Chandumpai et al., '91) and reproduction (Armitage et al., '72; Clarke, '79; Jeckel et al., '91). On the other hand, gill lipid concentration seems to be related to hypo-osmotic stress in both seasons. At this point, it is interesting to note that lipids not only play a metabolic role in providing energy for almost all endergonic processes, but they are also extremely important in maintaining the structural and physiological integrity of cellular and sub cellular membranes. During salinity adaptation, two types of mechanisms can be employed to maintain ionic hemolymph homeostasis: (1) 'limiting processes' acting on permeability properties of epithelial structures; and (2) 'compensatory processes' driving the active movement of water and ions. Both mechanisms are essentially linked to lipid metabolism. It is well known that phospholipids play an important role in membrane structure, which ultimately affects ion permeability. They also play a dynamic role in the function of membrane-bound proteins and can modulate enzymatic activity (Fourcans and Jain, '74; Sandermann, '78; Smith and Miller, '80). This is especially true for the Na^+ active transport, since Na^+,K^+-ATPase needs to be closely associated to phospholipids to accomplish its hydrolytic activity and, presumably, its ion transport function (Kimelberg and Papahadjopoulos, '74; Chapelle, '86). In euryhaline crustaceans, posterior gills (as opposed to anterior ones) have characteristic 'salt-transporting' epithelia, a physiological specialization that includes differences in ultrastructure of epithelial cells, modifications in shape and number of mitochondria, class and level of phospholipids, and enzyme activity (see Péqueux, '95, for review). Changes in number or activity of mitochondria might occur in response to increased energy requirement induced by the

augmented active transport of ions during adaptation to diluted media (Chapelle and Zwingelstein, '84). In both seasons, the reduction of gill total lipid content during hypo-osmotic stress could indicate that these compounds are serving as energy substrates. Also, this decrease in gill lipid concentration could be due to a possible tissue water gain during hypo-osmotic stress. However, the water gain (6.1‰) observed in gills after the hypo-osmotic shock in summer does not support this idea.

Another important point to consider is that gluconeogenesis could also play an important role in providing glucose and glycogen to satisfy crustacean metabolic requirements for carbohydrates. In fact, carbohydrate metabolism in the shrimp *Litopenaeus vannamei* is limited, and it is governed by protein metabolism (Rosas et al., 2001). After observing changes in the carbohydrate metabolism during osmoregulation in *C. granulata*, Nery and Santos ('93) suggested that lipids could be involved in the gluconeogenesis activity in summer. In fact, the relationship between the gluconeogenesis pathway and the adaptation to hypo-osmotic stress in *C. granulata* has already been demonstrated (Da Silva and Kucharski, '92; Oliveira and Da Silva, 2000). Furthermore, lipid involvement in gluconeogenic activity during osmotic stress, as proposed by Nery and Santos ('93), seems only to be occurring during the hypo-osmotic stress. This statement is based upon the fact that hemolymph and gill lipid mobilization only occurred after hypo-osmotic stress. Lipids, and possibly free amino acids, could be metabolized to provide glucose, since the amount of carbohydrates available in summer is critically low.

The hyper-osmotic shock did not induce lipid mobilization. From a broad view, total tissue lipids increased after the hype-osmotic shock. This fact could indicate that a different strategy is being employed during hyper-osmotic stress in *C. granulata*. In this context, protein metabolism may have a particular involvement in this situation. Several authors have studied the involvement of free amino acids (FAA) in crustacean osmoregulation. They have demonstrated that FAA are important osmotic effectors in cells of euryhaline species, and they could be considered as important osmolytes counteracting the effects of excessive hemolymph salt load. Abe et al. ('99) reported that some specific FAA significantly increased during acclimation of *Eriocheir japonicus* to artificial seawater. Similar results were found in shrimp

(*Palaemon elegans*, Dalla Via, '89, and *Macrobrachium rosenbergii*, Huong et al., 2001) acclimated to seawater.

Another hypothesis to be considered is that *C. granulata* is better adapted to support extremely high salinity (40‰) than fresh water. Mañe-Garzon et al. ('74) suggested that *C. granulata* is a sea-dependent species based on the fact that its ability to osmoregulate is of recent acquisition. Therefore, transference to extremely high salinity represents (in terms of gradient) a smaller osmotic work than transference to 0‰ salinity doses. The low mortality observed by Miranda ('94) during crab acclimation to hyper-osmotic media as opposed to hypo-osmotic media supports this hypothesis.

From a broad view, data presented here indicate that lipids constitute an energy store mobilized only during hypo-osmotic stress in *C. granulata*. Although hepatopancreas is the principal site of lipid store, there is no mobilization of lipids from this organ during *C. granulata* acclimation to hypo- or hyper-osmotic media.

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