Mini review

Usage of energy reserves in crustaceans during starvation: Status and future directions

Arturo Sánchez-Paz[a,b], Fernando García-Carreño[c], Adriana Muhlia-Almazán[b], Alma B. Peregrino-Uriarte[b], Jorge Hernández-López[b], Gloria Yepiz-Plascencia[b,*]

[a]*Centro de Investigaciones Biológicas del Noroeste (CIBNOR), Unidad Hermosillo, Centenario Norte #53 Col. Prados del Centenario, Hermosillo, Sonora C.P. 83260, Mexico*
[b]*Centro de Investigación en Alimentación y Desarrollo (CIAD), A.C., P.O. Box 1735, Carretera a la Victoria Km. 0.6 Hermosillo, Sonora C.P. 83000, Mexico*
[c]*Centro de Investigaciones Biológicas del Noroeste (CIBNOR), Mar Bermejo No. 195, Col. Playa Palo de Santa Rita, Apdo. Postal 128, La Paz, BCS C.P. 23090, México*

Abstract

In this paper, we review the current knowledge about the usage of carbohydrates, lipids and proteins as energy source by marine crustaceans during starvation. Crustaceans are a large and diverse group including some economically important species. The efforts to culture them for human consumption has prompted the interest to understand the preferences of energy sources to be applied for feed formulation and cost reduction. Important differences have been found among species and appear to be related not only to the biochemistry and physiology of nutrition, but also to the living environment of the crustaceans. Furthermore, crustaceans undergo morphological, physiological and behavioral changes due to their natural growing process that affect their feeding habits, an aspect that should be carefully considered. We discuss the current information on marine crustaceans about energy usage and describe areas of future research, where starvation studies render important insights.
© 2006 Elsevier Ltd. All rights reserved.

Keywords: Protein; Carbohydrate; Lipids; Starvation; Molt cycle; Crustaceans; Aquaculture

1. Introduction

Crustaceans are one of the most numerous and diverse group of non-insect arthropods. Most of the approximately 40,000 living species are marine, although some are found in freshwater and terrestrial environments. Due to their great diversity, crustaceans possess physiological and/or behavioral adaptation strategies to survive under a plethora of conditions and these adaptations may be specific for the species, feeding habits, gender, food availability and molting cycle (Muhlia-Almazán and García-Carreño, 2003). Comparative biochemistry in crustaceans has developed in recent years due to the interest in aquaculture, but has taken many initial hypotheses from the insect biochemistry field. Insects

are and will remain as a very useful model for biochemical research, and precisely due to its evolutionary closeness, crustacean biochemistry can take advantage of the available insect knowledge (Law and Wells, 1989).

A common denominator in crustaceans is their constant feeding activity (Cuzon et al., 2000). Furthermore, they alternate episodes of feeding and fasting during development, which occurs through molting (ecdysis) and results in growing by sequential steps. Increase in body size at each ecdysis is non-linear; this is a hormonally controlled process which might last days or weeks, is continuous and accompanied by morphological, physiological, and behavioral alterations occurring almost daily (Dall et al., 1990). Molting in crustaceans involves a series of stages with different feeding behavior and therefore, energy from food available. During intermolt, they feed actively; prior to molting, feeding declines until it stops completely during molting. Finally, feeding begins again in postmolt

when the animal has an exoskeleton rigid enough to support the weight of the animal and handle food (Phlippen et al., 2000).

The ability of an organism to survive and recover from long periods of starvation is vital. Changes in food intake during development may have important consequences for life history (Brzek and Konarzewski, 2001) and although, instantaneous ecological consequences of poor and spotty nutrition are sometimes difficult to distinguish, the reproductive potential of any organism experiencing such conditions may become reduced. Animals that experience natural cycles of food intake may have interesting adaptations for use and storage of energy sources.

Starvation can lead to a severe deficiency of nutrients. Therefore, starvation studies may be useful predictors to determine energetic and metabolic requirements (Guderley et al., 2003). Since crustaceans experience starvation periods during their growing process, artificially induced fasting and starvation may shed some light on the metabolic routes used—in hierarchical order—during molt and may describe novel biochemical and physiological adaptation mechanisms (Barclay et al., 1983). Furthermore, the knowledge derived from the understanding of their biochemical processes may be basis to optimize crustacean pond rearing efforts.

In this review, we focus on the data available concerning the main energy reserves used by crustaceans during starvation periods and the effects on the physiology of these organisms. Most of the available knowledge is based on studies of decapod crustaceans such as penaeids, crabs and lobsters that are economically important crustacean species.

2. Protein requirements

Proteins are critical for artificially reared crustaceans and are an expensive component of feeds for decapod crustaceans (Kureshy and Davis, 2000). Therefore, the dietary protein quantity and composition should be optimized to grant maximal growth (Shiau, 1998). Feed protein contents between 30% and 57% (w/w) are recommended for suitable growth for different species of penaeid shrimp (Córdova-Murueta and García-Carreño, 2002; Kureshy and Davis, 2000; Shiau, 1998). Differences in protein content required to yield similar growth rates are related to protein quality, age or physiological state of crustaceans (D'Abramo and Sheen, 1994). In addition, optimal dietary protein requirements can be influenced by the environment. Juvenile Penaeus monodon shrimp reared in seawater (40% protein) or 16 ppt brackish water (44% protein) (Shiau and Chou, 1991) had different growth rates. This can be due to differences in the use of dietary protein as energy source or to an effect on protein digestibility. Penaeus monodon acclimated to low salinity excreted more ammonium-N than those acclimated at high salinity, suggesting higher protein use under these conditions (Lei et al., 1989). Similarly, soybean digestibility was

lower in Penaeus monodon reared in brackish water (16 ppt) compared to sea water, but this lower salinity did not change the digestibility of casein and fish meal (Shiau et al., 1992).

2.1. Protein utilization during starvation

The understanding of crustacean nutritional requirements has increased over the last few decades; however, many gaps remain (Verri et al., 2001). Starvation studies give indications of the energy resources utilized by crustaceans under these conditions and provide clues to the biochemical pathways involved in these processes.

Several decades ago, it was proposed that in crustaceans, the primary source of energy is protein (New, 1976), contrasting with mammals and birds, which utilize mainly carbohydrates and lipids as energy source (Cherel et al., 1992). However, during a 28-day starvation study, in the hepatopancreas of the shrimp Penaeus japonicus, the glycogen stores were rapidly depleted, presumably being converted to glucose and used as energy source (Cuzon et al., 1980). Tail muscle lipids diminished progressively and proteins were next mobilized, but more slowly, eventually accompanied by muscular atrophy. Similar results were obtained for the shrimp Penaeus duorarum (Schafer, 1968) and for Crangon crangon (Cuzon and Ceccaldi, 1973). However, the purple shore crab Hemigrapsus nudus during a 23-day starvation period used preferentially protein (Neiland and Scheer, 1953) as reported for other decapods more recently (Anger, 2001). Moreover, crustacean responses to starvation appear to be influenced by the developmental stage. Spiny lobster Jasus edwardsii, phyllosoma larvae during a 6–11-day starvation catabolized more lipids than carbohydrates and proteins in stages II, IV and VI. These larvae were 14–40% lighter than their fed counterparts (Ritar et al., 2003).

In addition, changes may occur in which the crustacean switches to the use of one energy reserve to another, depending on the developmental stage. In the copepod Calanus finmarchicus, the use of energy changed: during the first 10 days of starvation the protein content showed a moderate decline, suggesting that this organism cope with starvation utilizing endogenous reserves different than protein; however, during the next 21 days, total protein content was drastically reduced (Helland et al., 2003). In subterranean aquatic crustaceans changes have also been found. After 28 days of starvation, the isopod Asellus aquaticus responded with an immediate, linear and large decrease of all the energy reserves, most of which were fully recovered after a 7-day refeeding period. In contrast, prolonged fasting (180 days) in the isopod Stenasellus virei was characterized by three successive phases: (1) an immediate, but low, depletion of both glycogen and arginine phosphate, followed by (2) the utilization of triacylglycerydes associated with glycogen resynthesis and finally (3) a slow depletion of both proteins (demonstrated by a slight increase in ammonia excretion rate) and lipids,

always associated with a glycogen resynthesis. As in *A. aquaticus*, *S. virei* energy reserves were fully recovered after a 15-day refeeding period (Hervant and Renault, 2002). Strategies of fuel reserves usage may change depending on the species and larval stage (Le Vay et al., 2001). Such strategies were favorably selected for the ability to prolong the survival of the organisms and, therefore, to increase their competitive abilities. The relative importance of metabolic reserves and their order of utilization vary among species (reviewed in Hervant et al., 1999).

3. Carbohydrates requirements

Carbohydrates are the most economical and inexpensive source of energy for crustacean feeds. Although not considered essentials, they are incorporated in aquaculture feeds to reduce costs and for their binding properties during feed manufacturing. Recent studies have reduced dietary protein by replacing it with carbohydrates, revealing that the ability of shrimp to utilize carbohydrates is limited as a consequence of both, the low storage capacity and the low capability of enzymatic processing (Rosas et al., 2000).

Even when carbohydrates are not essential for crustaceans, they can be a useful inexpensive source of energy with protein-sparing and lipid-sparing effects. *Penaeus monodon* shrimp fed starch or dextrin had significantly higher weight gain, feed efficiency ratio, protein efficiency ratio and survival than those fed glucose (Shiau and Peng, 1992). Hence, the required dietary protein level for *Penaeus monodon* is lower if starch, instead of glucose or dextrin, is used as a carbohydrate source. Based on these studies, starch is nowadays the typical carbohydrate in formulated feeds for crustaceans. It is well hydrolyzed by shrimp such as *Penaeus indicus* and *Penaeus vannamei*, but poorly digested by lobsters (Verri et al., 2001).

D-glucose in crustacean hemolymph comes from two main sources: from the direct absorption of dietary D-glucose through hepatopancreatic and intestinal epithelial cells, or from hepatopancreas, where it is stored as glycogen or synthesized by the gluconeogenic pathway. D-glucose levels in hemolymph are tightly controlled, particularly by the crustacean hyperglycemic hormone (CHH), a neuropeptide produced by the sinus gland of eyestalks (Verri et al., 2001). Besides its role on regulation of carbohydrate metabolism, CHH also plays significant roles in reproduction, molting and other physiological processes. If D-glucose levels drop, neurons of the sinus gland release CHH and this induces the hydrolysis of glycogen from muscle stores. On the contrary, if D-glucose concentration in hemolymph increases, CHH release is inhibited, reversing the process of D-glucose production in the hepatopancreas and muscle (Verri et al., 2001).

D-glucose levels in hemolymph are rigorously controlled and much lower than in mammals blood, with values of 0.9 ± 0.2 mM in the crayfishes *Procambarus clarkii*, and $0.03–0.19$ mM in *Orconectes limosus*; $0.1–0.3$ mM in the crabs *Carcinus maenas*, 0.8 mM in *Cancer pagurus* and $0.05–0.49$ mM in *Cancer borealis*; $0.77–1.39$ mM in the shrimp *Penaeus monodon*, and $1.1–1.4$ mM in the lobster *Homarus americanus* (Verri et al., 2001).

Glycogen is the primary fuel source in exercising crustaceans (Herreid and Full, 1988), and it is mainly stored on the hepatopancreas (Loret, 1993; Gibson and Barker, 1979), while it only represents near 1% of the muscle mass in the estuarine crab *Chasmagnathus granulata* (Nery et al., 1993). Glycogen has been postulated as the main source for the glucose necessary for chitin synthesis, a major component of crustaceans that may represent up to 35% of the dry weight of shrimp (Omondi and Stark, 1996; Abdel-Rahman et al., 1979).

3.1. Carbohydrates utilization during starvation

Besides the information previously provided on the preferential use of protein or other biomolecules by crustaceans under starvation, the information available is still scarce. The high protein requirement and the limited capacity of marine shrimp for lipid and carbohydrate storage (Dall and Smith, 1986) may be related to their ability to use protein as energy source for growth (Rosas et al., 2000). In general, simple sugars are poorly utilized by shrimp, although the mechanism responsible for this is not fully understood. One possibility may be saturation of absorption sites, inhibiting the assimilation of other nutrients as amino acids. Such effect has been observed in other organisms such as the rainbow trout (Hokaseno et al., 1979; Pieper and Pfeffer, 1980) and carp (Murai et al., 1983; Furuichi and Yone, 1982), an interaction not studied yet in penaeid shrimp (Shiau and Peng, 1992). Another suggested explanation is that dietary glucose is quickly absorbed and released into the hemolymph, resulting in a physiologically abnormal elevation of plasma glucose levels, triggering glucose excretion and, therefore, impairing its utilization as energy source (Shiau and Peng, 1992). This may argue about a preferential use of protein as the main energy supply rather than carbohydrates, which have limited storage and use.

Other factors may also affect the specific energy source used; for instance, parasites may trigger severe changes on the metabolism of their host. When the shrimp *Palaemonetes argentinus* is infected by the branchial ectoparasite isopod *Probopyrus ringueleti* and starved for 15 days, a decline in lipid concentrations and preferential carbohydrates utilization was detected (Neves et al., 2004). In unparasitized organisms no significant differences were observed on glucose and glycogen concentrations, while total lipids decreased significantly (nearly 66%) in the first 24 h, and remained low until the end of the experiment.

4. Lipid requirements

The information about the metabolic requirements of lipids in crustaceans is contrasting. While some authors

report that penaeid shrimp require dietary lipids to satisfy a variety of metabolic functions (González-Félix and Pérez-Velázquez, 2002), others point out that the shrimp may not have a definite lipid requirement (Shiau, 1998; D'Abramo, 1989). However, lipids are a major source of energy in marine invertebrates, including shrimp; furthermore, they are involved in several essential processes for their growth, molting and reproduction. Cell membrane structure depends largely on the combination of specific lipids and proteins. Additionally, lipid droplets accumulate in specific tissues serving as energy stores (Yepiz-Plascencia et al., 2000).

Cholesterol is an essential nutrient for crustaceans since they are incapable of de novo synthesis of the steroid ring (Rabid et al., 1999), it is also an important lipid class in mature shrimp ovaries, and is assumed to be an essential dietary lipid for shrimp maturation and reproduction (Wouters et al., 2001). Cholesterol is indeed known to meet several endocrine functions, and its mobilization during maturation was reviewed by Harrison (1990).

It has been demonstrated that shrimps have a limited ability to synthesize de novo the n-6 and n-3 families of fatty acids, including the polyunsaturated linoleic (18:2n-6, LOA) and linolenic (18:3n-3, LNA) acids. They also have a limited ability to elongate and desaturate these polyunsaturated fatty acids (PUFA) to highly unsaturated fatty acids (HUFA) such as arachidonic (20:4n-6, AA), eicosapentaenoic (20:5n-3, EPA) and docosahexaenoic (22:6n-3, DHA) acids. Consequently, these fatty acids are considered essential. Some combinations of n-3 and n-6 FA appear to be required in shrimp diets. Xu et al. (1994) observed greater growth in the shrimp *Farfantepenaeus chinensis* fed a diet with LOA and LNA each at 0.5% of feed, while Chandge and Paulraj (1998) made similar observations in *Farfantepenaeus indicus*. Moreover, a 2:3 ratio of LOA to LNA was optimum for *Penaeus monodon* (Glencross and Smith, 1999).

The importance of phospholipids (PLs) in penaeid shrimp nutrition has been demonstrated (González-Félix et al., 2002). PLs are components of membrane structures and mediators and modulators of transmembrane signaling, and act as emulsifiers facilitating the digestion and absorption of fatty acids, bile salts and other lipid-soluble materials. They are the main lipid class that is transported from one tissue or organ to another and may play specific roles for the transport of absorbed lipids from the gut epithelium into the hemolymph (Coutteau et al., 1997), of which, they are the main lipoproteic lipid component (Yepiz-Plascencia et al., 2000; González-Félix and Pérez-Velázquez, 2002).

As crustaceans eat, the lipids present in the feed are digested and absorbed through the digestive tract and transported to appropriate cells for storage or utilization. The main lipid storage organ in crustaceans is hepatopancreas. Lipids are mobilized to and from this organ through lipoproteins that bind and carry these hydrophobic molecules in the aqueous hemolymph environment. High-

density lipoproteins (HDL) and very high density lipoproteins (VHDL) are the main lipoproteins found in crustacean species (Lee and Puppione, 1978; Yepiz-Plascencia et al., 2000; Yepiz-Plascencia et al., 2002). Phosphatidylcholine is a particularly important PL because it is an essential component of these lipoproteins (Hertrampf, 1992).

One of the most important roles of lipids in crustaceans is related to reproduction, since they are associated with the maturation of oocytes and the survival of the initial larval stages. Free sterols are commonly found in developing ovaries, where they contribute to membrane structure and are precursors of hormones and steroids. A dramatic increase in ovarian lipid accumulation occurs during vitellogenesis, with lipids accounting for 18–41% (in various species) of the total ovarian dry mass at the end of ovarian maturation. In most species, PLs, triacylglycerols and sterols are the most abundant ovarian lipid classes. In the blue crab *Callinectes sapidus*, lipid droplets, which form a minor component in immature ovaries, constitute 27% of the total lipids in mature ovaries (Lee and Walker, 1995). The origin of lipids reaching the ovary is not fully understood. Lipids stored in the hepatopancreas have been shown to be transported to the ovary during vitellogenesis (for a review, see Harrison, 1990). However, the amount of lipids accumulated within the ovaries is greater than that stored in the hepatopancreas and therefore, lipids synthesis has been proposed to occur within the ovaries and developing oocytes. It was observed that female shrimp double their food consumption, indicating that lipids accumulating in the ovaries must originate from the food (Teshima et al., 1986). It is not known whether these lipids pass via the metabolic junction in the hepatopancreas or are taken up directly from the gut.

4.1. Lipid utilization during starvation

Reports about the metabolic requirements of protein and lipids under starvation in crustaceans are very contrasting. As mentioned before, several authors report protein as the main source of energy for starved crustaceans. However, according to Ritar et al. (2003), during starvation of crustaceans, at all life stages including larvae, there are three distinct phases of biomass degradation: at the beginning, energy-rich lipid reserves are preferentially mobilized, reflected in decreasing lipid:protein ratios, which is typical of short-term food deprivation. When much of the accessible lipid pool has been depleted, proteins are increasingly utilized. A significant part of the lipid pool is bound in crucial cell structures such as membranes, and, hence, is normally unavailable for energy metabolism. This phase of predominantly protein catabolism is indicated by an increase in the lipid:protein ratio and reflects the degradation of structures such as muscle and nervous tissue. In the final phase of starvation prior to death, structural lipids may also be degraded, so that the lipid:protein ratio decreases again. In this condition, the

larvae have passed their point-of-no-return and do not recover after refeeding.

Energy metabolism may suffer variations depending on the development stage. In the spiny lobster *J. edwardsii*, phyllosoma larvae starved during 6–11 days, lipids were the main energy source during food deprivation on late stages of development, while in stage I protein catabolism may be more important (Ritar et al., 2003). Differences in energy source usage may be species-specific, but a complicating fact when analyzing the available information is that, in several cases, not all the three energy sources (carbohydrates, proteins and lipids) were evaluated in the same crustacean under the same conditions. Starvation time, developmental stage and molting can have important effects.

In addition, the methodological approaches used for defining the reserves mobilized during starvation may vary. Johnston et al. (2004) examined the activities of four digestive enzymes in the phyllosoma larval stages I and IV of the spiny lobster as an indicator of nutrients preferentially employed by the organism during starvation. It was found that protease activities were significantly higher in both stages, suggesting that protein catabolism provided energy during food deprivation, while lipase activity decreased indicating that lipids may be spared for fueling later development stages. These results are contrasting with those reported by Ritar et al. (2003).

Among crustaceans, neutral lipids (mainly triacylglycerides, TG) are preferentially catabolized during starvation, while polar lipids (PL and cholesterol) are conserved due to their role as structural components of cell membranes (Hervant et al., 1999). In the tiger prawn *Penaeus*

esculentus, the abdominal 'muscle mass' is the main source for proteins and lipids during a 14-day starvation period (Barclay et al., 1983). Subterranean amphipods species (*Niphargus virei* and *N. rhenorhodanensis*), under a 180-day starvation, metabolize preferentially lipids (reaching 70% and 53% of the initial value) in order to save carbohydrates and phosphagen (the two main fermentable fuels metabolized during oxygen deficiency in crustaceans; Hervant et al., 1999) and to save muscular proteins. Therefore, these species could withstand a prolonged hypoxic period subsequent (or associated) to an initial nutritional stress, and could rapidly renew searching for food (i.e. an active habitat exploration, Danielopol et al., 1994).

5. Enzymes involved in the utilization of crustacean energy reserves

During fasting and starvation, crustaceans must use their energy reserves to meet their needs. This will involve the hydrolysis of proteins to amino acids, glycogen to glucose and TG to free fatty acids, by proteases, glycohydrolases and lipases, respectively (Fig. 1). The activity of these enzymes must be finely regulated to degrade the necessary energy reserves, preserving cell integrity as much as possible. Since the most important energy reserves in crustaceans are in hepatopancreas and muscle, the enzymes must be produced in these tissues. To our knowledge, information about the enzymes responsible for these hydrolytic processes in response to starvation in crustaceans is not yet available.

Crustacean hepatopancreas produces several digestive enzymes that are secreted for food digestion, including
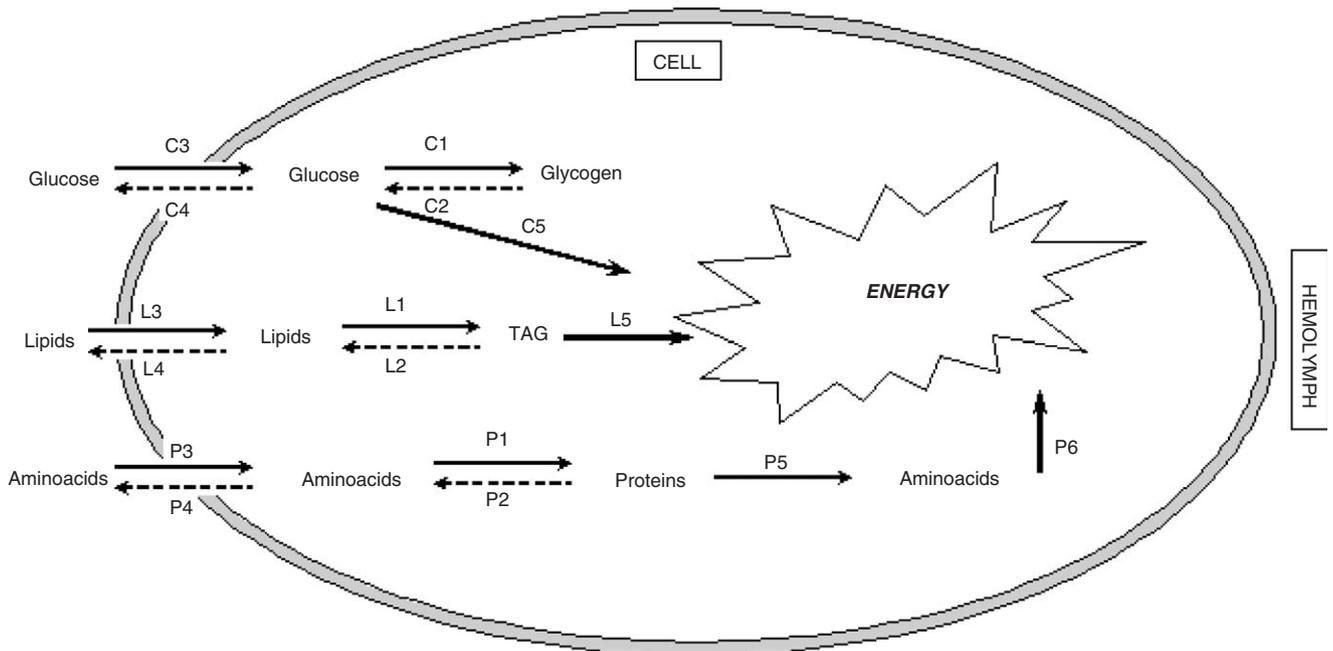

Fig. 1. A scheme showing the possible steps in a hepatopancreatic cell necessary to synthesize and use energy reserves. Energy is used intracellularly or released into the hemolymph to be taken up by other tissues. Continuous lines (C1, C3, C5, L1, L3, L5, P1, P3, P5 and P6) indicate synthesis, broken lines (C2, C4, L2, L4, P2, and P4) indicate degradation routes for energy reserves. C: carbohydrate, L: lipids, and P: Protein.

proteases like trypsin and chymotrypsin, lipases and carbohydrate degrading enzymes (Dall et al., 1990). Presumably, they are active only after secretion, stored as zymogens or in complexes with specific inhibitors (Neurath, 1984) that prevent internal cellular damage. During starvation, when cell internal reserves must be mobilized, it is possible that these same enzymes are produced or activated and finely regulated inside the cells; however, the processes and mechanisms whereby this occurs is still unknown, although some evidence for changes are becoming available.

Trysin and chymotrypsin activities (the main proteases) in the shrimp *Penaeus vannamei* hepatopancreas are 40–60% lower after 120 h starvation (Muhlia-Almazán and García-Carreño, 2002), while trypsin mRNA is ~30% lower (Sanchez-Paz et al., 2003), in agreement with their role as digestive enzymes that are secreted, and hence, if there is no food available, they appear to be down-regulated. If this trend is maintained after longer starvation periods is still unknown, but these proteases may be activated perhaps, once all the lipids are depleted, since the hepatopancreas is a lipid-rich tissue. The involvement of unidentified proteases is also possible.

Lipase activity has been recognized in crustaceans such as the Pacific white shrimp *Penaeus vannamei* (Gamboa-Delgado et al., 2003), redclaw crayfish *Cherax quadricarinatus* (López-López et al., 2003), the shrimp *Macrobrachium borellii* (González-Baro et al., 2000), and terrestrial isopods (Zimmer, 2002). However, the role and regulation of these enzymes under starvation periods is unknown. Besides, information related to genetic aspects of this kind of enzymes in crustaceans is null to our knowledge.

Crustacean digestive glycohydrolases are also reported on *Penaeus vannamei* (Van Wormhoudt and Sellos, 2003). Lovett and Felder (1990) found a substantial increase in amylase activity during postlarval development in *Penaeus*

setiferus as a response to low levels of carbohydrate in the diet. Similar results were found by Rodríguez et al. (1994) for myses of *Marsopenaeus japonicus* due to low dietary carbohydrate availability. Chitinase is also an important digestive enzyme (Lehnert and Johnson, 2002). However, their synthesis, regulation and expression during starvation in crustaceans are poorly understood. Moreover, there is no information to our knowledge about enzymes involved in glycogen degradation and their response to starvation. If the crustacean enzymes involved in energy reserves utilization are similar to insect enzymes, as expected by their closer phylogeny, remains to be elucidated. It is possible that some interesting characteristics and modes of regulations may be found in the enzymes from crustaceans.

6. Summary and concluding remarks

Studies about the metabolism of crustaceans and their ability to adapt to environmental variations contribute to the understanding and elucidation of perhaps new mechanisms. It is clear that some facts about protein metabolic requirements had been assumed or extrapolated to crustaceans, especially to penaeid shrimp, from the knowledge derived from other species. Therefore, more research is necessary to understand the biochemical and physiological aspects of crustacean nutritional requirements, especially considering the high degree of flexibility in digestive physiology of crustaceans, as an essential part of their ability to grow, survive, and reproduce when the food supply changes or depletes (Rodríguez et al., 1994; Icely and Nott, 1992). The metabolic requirements of crustaceans appear to be species-specific, and discrepancies may arise between phylogenetically related species. Table 1 presents some examples of the preferential use of energetic fuels by several species of crustaceans under food depletion. Starvation induction of crustacean in the intermolt

Table 1
Preferential usage of energy reserves of some crustaceans under prolonged fasting

Crustacean	Preferential energy supply depleted	Starvation period	Reference
Penaeus japonicus	Glycogen	28 days	Cuzon et al., 1980
Penaeus duorarum	Glycogen	—	Schafer, 1968
Crangon crangon	Glycogen		Cuzon and Ceccaldi, 1973
Palaemonetes argentinus	Carbohydrates	15 days	Neves et al., 2004
Stenasellus virei	Glycogen and arginine phosphate	120 days	Hervant and Renault, 2002
	Triacylglycerides		
	Proteins and lipids		
Calanus finmarchicus	Carbohydrates and lipids	First 10 days	Helland et al., 2003
	Protein	Next 21 days	
Penaeus esculentus	Proteins and lipids	14 days	Barclay et al., 1983
Jasus edwardsii[a]	Protein	8 days	Johnston et al., 2004
Hemigrapsus nudus	Protein	23 days	Neiland and Scheer, 1953
Jasus edwardsii[b]	Lipid	6–11 days	Ritar et al., 2003
Niphargus virei	Lipids	180 days	Hervant et al., 1999
Niphargus rhenorhodanensis	Lipids	180 days	Hervant et al., 1999

[a]Phyllosoma larval stages I and IV.
[b]Phyllosoma larval stages from hatching to stage VI.

stage is probably a good model to try to understand the molecular and enzymatic changes that occur naturally during their growing process, although the effect of hormones must not be forgotten.

Studies of energy reserves usage in fed, as well as in starved crustaceans, may be conducted by looking for changes in the enzymes responsible for their synthesis and degradation (Fig. 1). Current technology for identifying and characterizing genes expressed under specific conditions will also certainly be very useful and change the pace at which this information can be obtained. We believe that the understanding of the metabolic necessities of crustaceans, especially of those commercially important, will allow researchers to focus on more complicated issues, including the contributions of some other nutrients (such as proline, as in flying insects) to satisfy the energy requirements of organisms in undernourishment and how genes involved in energy metabolism respond to survive periods of starvation; this will eventually provide basis to propose strategies that could lead to a marked improvement in health and productivity of aquacultured crustaceans.

Acknowledgments

References

Abdel-Rahman, S.H., Kanazawa, A., Teshima, S., 1979. Effects of dietary carbohydrate on the growth and the level of the hepatopancreatic glycogen and serum glucose of prawn. Bull. Jpn. Soc. Sci. Fish. 45, 1491–1494.

Anger, K., 2001. The Biology of Decapod Crustacean Larvae. Swets & Zeitlinger, Lisse (420pp.).

Barclay, M.C., Dall, W., Smith, D.M., 1983. Changes in lipid and protein during starvation and the moulting cycle in the tiger prawn, Penaeus esculentus Haswell. J. Exp. Mar. Biol. Ecol. 68, 229–244.

Brzek, P., Konarzewski, M., 2001. Effect of food shortage on the physiology and competitive abilities of sand martin (Riparia riparia) nestlings. J. Exp. Biol. 204, 3065–3074.

Chandge, M.S., Paulraj, R., 1998. Requirements of linoleic and linolenic acid in the diet of Indian white prawn Penaeus indicus (H Milne Edwards). Indian J. Mar. Sci. 27, 402–406.

Cherel, Y., Robin, J.P., Heitz, A., Calgari, C., Le Maho, Y., 1992. Relationships between lipid availability and protein utilization during prolonged fasting. J. Comp. Physiol. 4B, 305–313.

Córdova-Murueta, J.H., García-Carreño, F.L., 2002. Nutritive value of squid and hydrolyzed protein supplement in shrimp feed. Aquaculture 210, 371–384.

Coutteau, P., Geurden, I., Camara, M.R., Bergot, P., Sorgeloos, P., 1997. Review on the dietary effects of phospholipid in fish and crustacean larviculture. Aquaculture 155, 149–164.

Cuzon, G., Ceccaldi, H.J., 1973. Influence of the fasting stabulation on the metabolism of the shrimp Crangon crangon (L.). C. R. Soc. Biol. 167, 66–69.

Cuzon, G., Cahu, C., Aldrin, J.F., Messager, J.L., Stephan, G., Mevel, M., 1980. Starvation effect on metabolism of Penaeus japonicus. Proc. World Maricult. Soc. 11, 410–423.

Cuzon, G., Rosas, C., Gaxiola, G., Taboada, G., Van Wormhoudt, A., 2000. Utilization of carbohydrates by shrimp. In: Cruz-Suárez, L.E., Ricque-Marie, D., Tapia-Salazar, M.A., Civera-Cerecedo, R. (Eds.), Avances en Nutrición Acuícola V. Memorias del V Simposium de Nutrición Acuícola. 19–22 Noviembre, 2000. Mérida, Yucatán. pp. 328–339.

D'Abramo, L.R., 1989. Lipid requirements of shrimp. Advances in Tropical Aquaculture. AQUACOP-IFREMER, Tahiti, pp. 271–285.

D'Abramo, L.R., Sheen, S.S., 1994. Nutritional requirements, feed formulation, and feeding practices for intensive culture of the freshwater prawn Macrobrachium rosenbergii. Rev. Fish. Sci. 21, 504–515.

Dall, W., Smith, D.M., 1986. Oxygen consumption and ammonia-N excretion in fed and starved tiger prawns Penaeus esculentus Haswell. Aquaculture 55, 23–33.

Dall, W., Hill, B.J., Rothlisberg, P.C., Sharples, D.J., 1990. Moulting and growth. In: Blaxter, J.H.S., Southward, A.J. (Eds.), The Biology of the Penaeidae. Advances in Marine Biology, vol. 27. Academic Press, London, pp. 213–250.

Danielopol, D.L., Creuzé des Châtelliers, M., Mösslacher, F., Pospisil, P., Popa, R., 1994. Adaptation of Crustacea to interstitial habitats: a practical agenda for ecological studies. In: Gibert, J., Danielopol, D.L., Stanford, J.A. (Eds.), Groundwater Ecology. Academic Press, San Diego, pp. 217–243.

Furuichi, M., Yone, Y., 1982. Availability of carbohydrate in nutrition of carp and red sea bream. Bull. Jpn. Soc. Sci. Fish. 48, 945–948.

Gamboa-Delgado, J., Molina-Poveda, C., Cahu, C., 2003. Digestive enzyme activity and food ingesta in juvenile shrimp Litopenaeus vannamei (Boone, 1931) as a function of body weight. Aquacult. Res. 15, 1403–1411.

Gibson, R., Barker, P.L., 1979. The decapod hepatopancreas. Oceanogr. Mar. Biol.: Annu. Rev. 17, 285–346.

Glencross, B.D., Smith, D.M., 1999. The dietary linoleic and linolenic fatty acids requirements of the prawn Penaeus monodon. Aquacult. Nutr. 5, 53–63.

González-Baro, M.R., Heras, H., Pollero, R.J., 2000. Enzyme activities involved in lipid metabolism during embryonic development of Macrobrachium borellii. J. Exp. Zool. 286, 231–237.

González-Félix, M.L., Pérez-Velázquez, M., 2002. Current status of lipid nutrition of Pacific white shrimp, Litopenaeus vannamei. In: Cruz-Suárez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Gaxiola-Cortés, M.G., Simoes, N. (Eds.), Avances en Nutrición Acuícola VI. Memorias del VI Simposium Internacional de Nutrición Acuícola. 3 al 6 de Septiembre del 2002. Cancún, Quintana Roo, México.

González-Félix, M.L., Lawrence, A.L., Gatlin III, D.M., Pérez-Velázquez, M., 2002. Growth, survival and fatty acid composition of juvenile Litopenaeus vannamei fed different oils in the presence and absence of phospholipids. Aquaculture 205, 325–343.

Guderley, H., Lapointe, D., Bedard, M., Dutil, J.D., 2003. Metabolic priorities during starvation: enzyme sparing in liver and white muscle of Atlantic cod, Gadus morhua L. Comp. Biochem. Physiol. 2A, 347–356.

Harrison, K.E., 1990. The role of nutrition in maturation, reproduction and embryonic development of decapod crustaceans: a review. J. Shellfish Res. 9, 1–28.

Helland, S., Nejstgaard, J.C., Fyhn, H.J., Egge, J.K., Bamstedt, U., 2003. Effects of starvation, season, and diet on the free amino acid and protein content of Calanus finmarchicus females. Mar. Biol. 143, 297–306.

Herreid, C.F., Full, R.J., 1988. Energetics and locomotion. In: Burggren, W.W., McMahon, B.R. (Eds.), Biology of the Land Crabs. Cambridge University Press, New York, pp. 333–377.

Hertrampf, W.J., 1992. Feeding Aquatic Animals with Phospholipids II. Fishes. Lucas Meyer Publication No. 11. Lucas Meyer GmbH & Co., Hamburg (70pp.).

Hervant, F., Renault, D., 2002. Long-term fasting and realimentation in hypogean and epigean isopods: a proposed adaptive strategy for groundwater organisms. J. Exp. Biol. 205, 2079–2087.

Hervant, F., Mathieu, J., Barre, H., 1999. Comparative study on the metabolic responses of subterranean and surface-dwelling amphipod crustaceans to long-term starvation and subsequent refeeding. J. Exp. Biol. 202, 3587–3595.

Hokaseno, S., Tanska, Y., Katayama, T., Chichester, C.O., Simpson, K.L., 1979. Intestinal transport of L-lysine in rainbow trout, *Salmo gairdneri*. Nippon Suisan Gakkaishi 45, 845–848.

Icely, J.D., Nott, J.A., 1992. Digestion and absorption: digestive system and associated organs. In: Harrison, F.W. (Ed.), Microscopic Anatomy of Invertebrates, vol. 10. Wiley, New York, pp. 147–202.

Johnston, D.J., Ritar, A.J., Thomas, C.W., 2004. Digestive enzyme profiles reveal digestive capacity and potential energy sources in fed and starved spiny lobster (*Jasus edwardsii*) phyllosoma larvae. Comp. Biochem. Physiol. 138B, 137–144.

Kureshy, N., Davis, D.A., 2000. Metabolic requirements for protein by pacific white shrimp, *Litopenaeus vannamei*. In: Cruz-Suárez, L.E., Ricque-Marie, D., Tapia-Salazar, M.A., Civera-Cerecedo, R. (Eds.), Avances en Nutrición Acuícola V. Memorias del V Simposium de Nutrición Acuícola. 19–22 Noviembre, 2000. Mérida, Yucatán. pp. 161–180.

Law, J.H., Wells, M.A., 1989. Insects as biochemical models. J. Biol. Chem. 264, 16335–16338.

Lee, R.F., Puppione, D.L., 1978. Serum lipoproteins in the spiny lobster, *Panulirus interruptus*. Comp. Biochem. Physiol. 59B, 239–243.

Lee, R.F., Walker, A., 1995. Lipovitellin and lipid droplet accumulation in oocytes during ovarian maturation in the blue crab, *Callinectes sapidus*. J. Exp. Zool. 271, 401–412.

Lehnert, S.A., Johnson, S.E., 2002. Expression of hemocyanin and digestive enzyme messenger RNAs in the hepatopancreas of the Black Tiger Shrimp *Penaeus monodon*. Comp. Biochem. Physiol. 133B, 163–171.

Lei, C.H., Hsieh, L.Y., Chen, C.K., 1989. Effects of salinity on the oxygen consumption and ammonia-N excretion of young juvenile of the grass shrimp, *Penaeus monodon* Fabricius. Bull. Inst. Zool. Acad. Sin. 28, 245–256.

Le Vay, L., Jones, D.A., Puello-Cruz, A.C., Sangha, R.S., Ngamphongsai, C., 2001. Digestion in relation to feeding strategies exhibited by crustacean larvae. Comp. Biochem. Physiol. 128A, 623–630.

López-López, S., Nolasco, H., Vega-Villasante, F., 2003. Characterization of digestive gland esterase-lipase activity of juvenile redclaw crayfish *Cherax quadricarinatus*. Comp. Biochem. Physiol. 135B, 337–347.

Loret, S.M., 1993. Hemocyte differentiation in the shore crab *Carcinus maenas* could be accompanied by a loss of glycogenosynthesis capability. J. Exp. Zool. 167, 548–555.

Lovett, D.L., Felder, D.L., 1990. Ontogenetic change in digestive enzyme activity of larval and postlarval white shrimp *Penaeus setiferus* (Crustacea, Decapoda, Penaeidae). Biol. Bull. 178, 144–159.

Muhlia-Almazán, A., García-Carreño, F.L., 2002. Influence of molting and starvation on the synthesis of proteolytic enzymes in the midgut of the white shrimp *Penaeus vannamei*. Comp. Biochem. Physiol. 133, 383–394.

Muhlia-Almazán, A., García-Carreño, F.L., 2003. Digestion physiology and proteolytic enzymes of crustacean species of the Mexican Pacific Ocean. In: Hendrickx, M.E. (Ed.), Contributions to the Study of East Pacific Crustaceans, vol. 2. Instituto de Ciencias del Mar y Limnología, UNAM, pp. 77–91.

Murai, T., Akiyama, T., Nose, T., 1983. Effect of glucose chain length of various carbohydrates and frequency of feeding on their utilization by fingerling carp. Nippon Suisan Gakkaishi 49, 1607–1611.

Neiland, K.A., Scheer, B.T., 1953. The influence of fasting and of sinus gland removal on body composition of *Hemigrapsus nudus*. Physiol. Comp. Oecol. 3, 321.

Nery, L.E., Santos, E.A., Bianchini, A., Goncalves, A.A., 1993. Effects of crustacean hyperglycemic hormones from *Carcinus maenas* and *Orconectes limosus* on blood and muscle glucose and glycogen concentration of *Chasmagnathus granulata*. Braz. J. Med. Biol. Res. 26, 1291–1296.

Neurath, H., 1984. Evolution of proteolytic enzymes. Science 224, 350–357.

Neves, C.A., Pastor, M.P., Nery, L.E., Santos, E.A., 2004. Effects of the parasite *Probopyrus ringueleti* (Isopoda) on glucose, glycogen and lipid concentration in starved *Palaemonetes argentinus* (Decapoda). Dis. Aquat. Organ. 2–3, 209–213.

New, M.B., 1976. A review of dietary studies with shrimp and prawns. Aquaculture 9, 101–144.

Omondi, J.G., Stark, J.R., 1996. In vitro carbohydrate digestibility tests in the Indian white shrimp, *Penaeus indicus*. Aquaculture 139, 315–328.

Phlippen, M.K., Webster, S.G., Chung, J.S., Dircksen, H., 2000. Ecdysis of decapod crustaceans is associated with a dramatic release of crustacean cardioactive peptide into the haemolymph. J. Exp. Zool. 203, 521–536.

Pieper, A., Pfeffer, E., 1980. Studies on the comparative efficiency of utilization of gross energy from some carbohydrates, protein and fats by rainbow trout (*Salmo gairdneri*). Aquaculture 20, 323–332.

Rabid, T., Tietz, A., Khayat, M., Boehm, E., Michelis, R., Lubzens, E., 1999. Lipid accumulation in the ovaries of a marine shrimp *Penaeus semisulcatus* (de haan). J. Exp. Biol. 202, 1819–1829.

Ritar, A.J., Dunstan, G.A., Crear, B.J., Brown, M.R., 2003. Biochemical composition during growth and starvation of early larval stages of cultured spiny lobster (*Jasus edwardsii*) phyllosoma. Comp. Biochem. Physiol. 136A, 353–370.

Rodríguez, A., Le Vay, L., Mourente, G., Jones, D.A., 1994. Biochemical composition and digestive enzyme activity in larvae and postlarvae of *Penaeus japonicus* during herbivorous and carnivorous feeding. Mar. Biol. 118, 45–51.

Rosas, C., Cuzon, G., Gaxiola, G., Pascual, C., Brito, R., Chimal, M. E., Van Wormhoudt, A., 2000. El metabolismo de los carbohidratos de *Litopenaeus setiferus*, *L. vannamei*, y *L. stylirostris*. In: Cruz-Suárez, L.E., Ricque-Marie, D., Tapia-Salazar, M.A., Civera-Cerecedo, R. (Eds.), Avances en Nutrición Acuícola V. Memorias del V Simposium de Nutrición Acuícola. 19–22 Noviembre, 2000. Mérida, Yucatán. pp. 340–359.

Sanchez-Paz, A., García-Carreño, L.F., Muhlia-Almazán, A., Hernández-Saavedra, N., Yepiz-Plascencia, G., 2003. Differential expresión de trypsin mRNA in the white shrimp (*Penaeus vannamei*) midgut gland under starvation conditions. J. Exp. Mar. Biol. Ecol. 292, 1–7.

Schafer, H.J., 1968. Storage materials utilized by starved pink shrimp, *Penaeus duorarum* Burkenroad. FAO Fish. Rep. 57, 393–403.

Shiau, S.Y., 1998. Nutrient requirements of penaeid shrimpsshrimp. Aquaculture 164, 77–93.

Shiau, S.Y., Chou, B.S., 1991. Effects of dietary protein and energy on growth performance of tiger shrimp *Penaeus monodon* reared in seawater. Nippon Suisan Gakkaishi 57, 2271–2276.

Shiau, S.Y., Peng, C.Y., 1992. Utilization of different carbohydrates at different dietary protein levels en grass prawn, *Penaeus monodon*, reared in seawater. Aquaculture 101, 241–250.

Shiau, S.Y., Lin, K.P., Chiou, C.L., 1992. Digestibility of different protein sources by *Penaeus monodon* raised in brackish water and in seawater. J. Appl. Aquacult. 3, 47–53.

Teshima, S., Kanazawa, A., Kakuta, Y., 1986. Role of dietary phospholipids in the transport of [14C]tripalmitin in the prawn. Bull. Jpn. Soc. Sci. Fish. 52, 519–524.

Van Wormhoudt, A., Sellos, D., 2003. Highly variable polymorphism of the alpha-amylase gene family in *Litopenaeus vannamei* (Crustacea Decapoda). J. Mol. Evol. 57, 659–671.

Verri, T., Mandal, A., Zilli, L., Bossa, D., Mandal, P.K., Ingrosso, L., Zonno, V., Vilella, S., Ahearn, G.A., Storelli, C., 2001. D-Glucose transport in decapod crustacean hepatopancreas. Comp. Biochem. Physiol. 130A, 585–606.

Wouters, R., Lavens, P., Nieto, J., Sorgeloos, P., 2001. Penaeid shrimp broodstock nutrition: an updated review on research and development. Aquaculture 202, 1–21.

Xu, X., Wenjuan, J., Castell, J.D., O'Dor, R., 1994. Essential fatty acid requirement of the Chinese prawn, *Penaeus chinensis*. Aquaculture 127, 29–40.

Yepiz-Plascencia, G., Vargas-Albores, F., Higuera-Ciapara, I., 2000. Penaeid shrimp hemolymph lipoproteins. Aquaculture 191, 177–189.

Yepiz-Plascencia, G., Jiménez-Vega, F., Romo-Figueroa, M.G., Sotelo-Mundo, R.R., Vargas-Albores, F., 2002. Molecular characterization of the bifunctional VHDL-CP from the hemolymph of the white shrimp *Penaeus vannamei*. Comp. Biochem. Physiol. 132B, 585–592.

Zimmer, M., 2002. Nutrition in terrestrial isopods (Isopoda: Oniscidea): an evolutionary-ecological approach. Biol. Rev. Cambridge Philos. Soc. 77, 455–493.