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SOME NUTRITIONAL AND BIOCHEMICAL ASPECTS FOR DEVELOPMENTAL STUDIES OF PENAEID SHRIMPS IN CULTURE.

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ABSTRACT

Several penaeid species present a "critical period" during development in culture which is marked by high mortality of organisms. Studies have shown that larval and early postlarval stages are characterized by this problem, whose causes are still unknown. In the quest to better understand this question, looking for high production levels, techniques previously used on studies of secondary production can help as a valid tool. Nutritional and biochemical studies on the cultured shrimp and the food supplied can determine the most important constituents for a successfull developmental period. Quantitative aspects like ingestion and food conversion must also be considered.

The present paper describes and evaluates four different methods used in the study of decapod crustacean development that can be applied to penaeid species: studies on elemental and biochemical composition of the food provided to larvae and postlarvae; ingestion of shrimps fed on live food; digestive enzyme activity, and elemental and biochemical composition of cultured organisms.

Literature data confirm the utility of these methods for penaeid developmental studies and culture.

Keywords: Penaeid shrimp, culture, nutrition, biochemistry, ingestion, development.

INTRODUCTION

Despite largely studied during the last two decades, larval and postlarval development of penaeid shrimps in culture still presents "critical phases", which are characterized by high mortality, specially in certain stages of the life cycle. First, many authors considered zoea (Hudinaga, 1942; Mock and Neal, 1974), while more recently the early postlarval period has been recognized by high mortality of organisms (Rodríguez, 1976; Wickins, 1976; Bages and Sloane, 1981). It is known that penaeid shrimp postlarvae exhibit many kinds of changes during transition to postlarva. Their feeding habits uses to modify considerably from planktonic to benthonic life (Gleason and Zimmerman, 1984; Gleason, 1986) and digestive anatomy and physiology also undergo significant changes (Lovett and Felder, 1989; 1990a; 1990b; Abubakr and Jones, 1992). The study of digestive enzyme activity during development in culture could be used to verify if changes are related to food quality and availability or just with ontogeny.

Furthermore, feed is a major expense in shrimp farming. It is in the order of 55% of total variable costs (Hardman *et al.*, 1991), a situation that leads to a considerable interest in alternative diets, with lower costs, that keep high productivity in culture (Sarac *et al.*, 1993). Much has been done on the composition of feeds employed in penaeid farming as well as on the nutritional requirements of the species for diet compounds (see Akiyama *et al.*, 1992). On the other hand, only few works (see Cruz-Ricque *et al.*, 1989; Lim and Hirayama, 1993; Le Vay *et al.*, 1993; Rodríguez *et al.*, 1994) examine the effect of these feeds on the body composition of shrimps, their assimilation and the importance of some body constituents for the animal to succeed at each stage of development.

The present paper explain some techniques applied in a nutritional study: digestive enzyme activity, feed and cultured organisms composition, and ingestion quantification. Based on literature data, it discusses the use of these analysis for a better understanding of feeding, survival, and growth of penaeids in culture. In addition, some hypothesis about the digestive physiology of shrimps are presented, and the utility of these methods as a tool for improving rearing practices evaluated.

MATERIAL AND METHODS

Larvae and postlarvae of penaeids are generally acquired in shrimp farming facilities or through breeding of ripe females caught in the natural environment. A great number of cultured individuals are necessary for this kind of study due to the amount of material involved in elemental, biochemical and enzymatic analysis. The aim of these techniques is to study survival and growth of cultivated organisms during development by means of analysis of body and feed composition. It makes possible to compare the suitability of different diets to production or to examine changes in organisms submitted to quali-quantitative variations of diet.

Techniques

1) Digestive enzyme activity: Samples (fresh larvae or postlarvae) must be kept at least at -20 ^{o}C before analysis. The homogenates (Sample+buffer) are centrifuged, and the supernatant is collected for the following assays.

Amylase (Rick and Stegbauer, 1984): Samples of the supernatant are incubated with 1% w/v starch solution in a phosphate buffer. Production of reducing sugars is quantified colorimetrically following addition of dinitrosalicylic acid reagent. A standard curve is prepared using maltose solution instead of enzyme sample, and results are expressed as moles of maltose equivalents released per minute.

Trypsin (Rick, 1984): Trypsin-like activity is determined using N - p - toluenesulphonyl - L - argenine methyl ester (TAME) as substrate. The rate of

hydrolisis of the substrate is recorded as increase in absorbance at 247 nm. Activity is calculated and expressed in units of moles of substrate cleaved per minute, based on a extinction coefficient of 0.54 cm^2. mol^{-1} for the product of reaction.

Cholinesterase (Ellman *et al.*, 1961; Whittaker, 1984): Activity is determined using 5-5' - diothio - bis (2 - nitrobenzoic acid) (DTNB) as substrate. The rate of hydrolisis of the substrate is measured spectrophotometrically at 410 nm. Based on a extinction coefficient of 1.36 cm^2.mol^{-1} for the product of reaction, calculation is as for trypsin.

Total activity can be divided either by the number of larvae in the sample to give activity per individual, or by protein content to give specific activity.

Lovett and Felder (1990a) presented a good review on enzymes and substrates tested on penaeid shrimps. More details on the analytical routine are described by Le Vay *et al.* (1993) and Rodríguez *et al.* (1994).

2) Elemental and biochemical composition of feed and cultured shrimps (all samples must be dry matter):

Elemental composition (C, N, H): Most studies are carried out by using an elemental analyzer (for example: Carlo Erba 1106, with cyclohexane 2,4 - dinitrophenylhydrozone as standard) and results are expressed as percentage of each element. Through sample dry weight it is possible to estimate the relative amounts of carbon, nitrogen and hydrogen. The energetic content can be obtained by multiplying carbohydrate, protein and lipid contents by 17.14, 23.42, 39.31 J, respectively (Winberg, 1971) or simply estimated from carbon content (Salonen *et al.*, 1976).

Biochemical composition: Sample composition is determined by following the dye binding method (Kochert, 1978a) for proteins, and the phenol-sulphuric acid method for carbohydrates contents (Kochert, 1978b). Lipid content can be quantified by the gravimetric method of Folch *et al.* (1957).

3) Ingestion: Larvae and postlarvae can be fed on live food (i. e., microalgae, rotifers and *Artemia* sp.) in order to determine the ingestion rate, survival and weight gain (dry matter). This study can also compare the efficacy of different kinds of food provided to shrimps. The incubation method is a reliable technique, where predators (shrimps) are confined in a container and measurements of the change in prey concentration are registered after a certain period of incubation time. The experimental procedure may follow previous works as Paffenhofer (1971), Emmerson (1980, 1984), Yufera *et al.* (1984) and Loya-Javellana (1989). When studying ingestion on microalgae it becomes crucial to determine algal growth rates, and some artifices to diminish sources of errors in these experiments were described by Saiz (1993).

RESULTS

The techniques described above have been generally applied in studies that are not necessarily related with aquaculture. The results presented here are a compilation of works on many crustacean genera, in order to describe physiological and behavioural changes during their development.

A synthesis of many works on enzyme activity through the development of penaeid shrimps in culture is shown in Table 1. With the exception of *Penaeus setiferus* (Lovett and Felder, 1990a), other studies refer to *Penaeus japonicus* (Laubier-Bonichon *et al.*, 1977; Galgani *et al.*, 1985; Le Vay *et al.*, 1993; Rodríguez *et al.*, 1994). These results show that there seems to be no pattern of activity for a same species through its development, despite its similar diet in culture (see Laubier-Bonichon *et al.*, 1977; Rodríguez *et al.*, 1994). They also show that activity peaks generally occur at larval stages, specially zoea (Galgani *et al.*, 1985; Lovett and Felder, 1990a; Le Vay *et al.*, 1993), decreasing towards the first postlarvae. Through postlarval development, activity tends to be low with the exception of amylase in *Penaeus setiferus* (Lovett and Felder, 1990a).

Studies on elemental and biochemical composition and energy content of penaeid shrimps in culture are still scarce. Results for many crustacean species are presented in Table 2. Notwithstanding the great variety of genera presented, it is possible to identify defined patterns for some constituents. Energy content tends to decrease during ecdysis and towards the postlarval phase. The same pattern is valid for C:N ratio which tends to increase in post moult and decrease in pre moult. Although registered by a single study (see Anger, 1987), lipid and protein contents also decrease during the moult cycle. The changes in body constituents among these species show the former are consumed towards ecdysis.

The ingestion rate, at least during larval development of shrimps, varies in accordance with food availability. The relationship between these topics for many shrimp species in culture is shown in Table 3. In the majority of studies, ingestion rate is limited above a certain concentration of food (asymptotic relationship). On the other hand, many penaeids (*P. marginatus* and *P. kerathurus*) do not present this limitation, ingestion being a direct function of food availability (linear relationship). This fact shows that differences in ingestion behaviour occur between species from a same generus (*Penaeus*).

DISCUSSION

Patterns of digestive enzyme activity in penaeids and other crustaceans through development have been explained by ontogenetic and dietary hypothesis. Many authors (see: Samain *et al.*, 1980; Lovett and Felder, 1990a) consider that changes in enzyme activity depend only on ontogenetic changes of digestive apparatus of the organisms. In penaeids, the decrease in activity after metamorphosis to postlarva may be due to the degeneration of the larval

digestive system (specially the anterior midgut diverticulum) in the transition from planktonic to benthonic habits (Lovett and Felder, 1989). In this phase, the retention time of food in the gut is short, and the digestive system is not totally transformed and functional if compared to a 4-5 week postlarvae, which presents a markedly higher activity (Lovett and Felder, 1989; 1990a). The metabolic activity also decreases in early postlarvae (Laubier-Bonichon *et al.*, 1977) what is believed to be the "critical point" of development (Rodríguez, 1976; Wickins, 1976; Bages and Sloane, 1981). In wild populations, a more substantial carnivorous feeding occurs in late postlarvae, PL_{28-35} (Hudinaga, 1969; Gleason and Zimmerman, 1984), while in culture organisms are able to survive to postlarva feeding only on microalgae (Rodríguez *et al.*, 1994).

Changes in digestive physiology can also be related to quali-quantitative aspects of culture. Certain studies try to establish a relationship between variations in enzyme activity and changes in nutritional requirements of shrimps, aiming to determine a suitable diet for each phase of rearing (Van Wormhoudt, 1973; Laubier-Bonichon *et al.*, 1977; Lee and Lawrence, 1985). Thus, the increase in activity of a digestive enzyme could maximize the utilization of a scarce component in the diet (Hernandorena, 1982; Hofer, 1982; Harris *et al.*, 1986). Else, Le Vay *et al.* (1993) and Rodríguez *et al.* (1994) pointed out that the supply of microalgae during all larval stages could cause a stimulation of enzyme secretion and a better use of the food offered (*e.g. Artemia*, rotifers, artificial feed), with algae having a "beneficial influence" on food assimilation. On the other hand, the amount of food provided and the enzyme activity can show positive (Boucher and Samain, 1974; Mayzaud and Poulet, 1978), negative (Cox and Willason, 1981; Moal *et al.*, 1981; Hirche, 1989) or no correlation (Head and Conover, 1983; Head *et al.*, 1984), which are questions still not totally understood. Special attention must be payed to these correlations in the study of the effect of diet in enzyme activity and assimilation.

The validity of these studies and hypothesis depends on the fact that enzyme activity may be studied on constant nutritional and physiological state of the cultured organisms. The stage of molt cycle, nutritional status, sexual condition, season, and ontogenetic stage affect the body composition of shrimps (Cuzon *et al.*, 1980; Pascual *et al.*, 1983; Lee *et al.*, 1984; Vogt *et al.*, 1985; Mourente *et al.*, 1990: Mourente and Rodríguez, 1991). As the majority of enzyme activity representations are based on body composition (i.e., IU/mg of Dry Weight or IU/mg of Total Protein), changes in activity are necessarily related to the factors cited above (Van Wormhoudt *et al.*, 1980; Barclay *et al.*, 1983; Lee *et al.*, 1984; Lovett and Felder, 1990a) what must be considered for any final conclusion.

The bulk of publications about body composition of decapod crustaceans in culture deal with genera other than penaeids (*Hyas araneus*: Anger *et al.*, 1983; Anger, 1987; Harms *et al.*, 1991. *Inachus dorsettensis*: Anger, 1988. *Pagurus bernhardus*: Dawirs, 1980. *Carcinus maenas*: Dawirs, 1980; 1987; Dawirs *et al.*, 1986. *Tisbe holothuriae*: Zhang and Uhlig, 1993; see also Table 2). In these studies, changes in C:N ratio, protein, lipid and energy contents

are analysed so that patterns of development in the organisms which varies according to culture conditions (temperature, salinity and diet), can be determined. The use of these parameters throughout a moult cycle, for example, allows to identify which are the most important body constituents for the animal to succeed at each stage of development (Anger *et al.*, 1983). When these requirements are associated with absortion, retention and conversion of dietary nutrients, the nutritional status of the organism can be understood integrally, and better culture conditions can be established. In this kind of experiment, survival rate may be a useful indicator of shrimps' condition. Energy content can also be employed as a feed condition index while explains if energy balance is positive or negative. This parameter can be used to verify the suitability of a certain diet or the effect of culture conditions, as for example, temperature (see: Anger, 1987).

Besides the qualitative aspects of penaeid nutrition, the correct quantification of ingested food is also important for a satisfactory development of shrimp in culture. Many rearing facilities still adopt feeding protocols largely based just on empirical observations in tanks and survival, but these do not always reflect the actual needs of the organisms (Loya-Javellana, 1989). Else, under excessive feeding, live food would not only compete with shrimp for dissolved oxigen, but also add metabolic wastes to the culture facility (Chu and Shing, 1986). The studies about ingestion of shrimps on live food show that it is mainly conditioned by food availability (see Table 3). Furthermore, in determining the optimal qualitative aspects of the diet, it is important to consider the amount of food caught and its efficiency. Studies on ingestion of penaeids submitted to increasing concentrations of food make it possible to recognize the incipient limiting level (ILL), defined as the lowest food density to provide maximum ingestion rates (McMahon and Rigler, 1963), another important information to set optimal culture conditions.

Since 1985, shrimp catches reduced drastically in Brazil (FAO, 1994). As alternatives to enhance production, pond cultivation and releasing programmes showed sactisfactory results in many countries (Kittaka, 1981; Lester, 1992). However, studies on the performance of Brazilian native species in culture are not abundant despite their importance for the purposes cited above. Thus, the techniques here presented could be useful instruments in the quest to produce postlarvae of shrimp under controlled conditions.

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Table 1. Some studies on digestive enzyme activity through the development of penaeid shrimps.

Species	Remarks	Reference
Penaeus japonicus	Amylase and trypsin activities rise in Z1, reaching their maximum in M1 and decreasing towards PL1. Postlarval period: amylase shows a slight enhancement 'till PL20 while trypsin keeps low. Diet: microalgae, rotifers, *Artemia* nauplii and mussel.	Laubier-Bonichon *et al.*, 1977.
Penaeus japonicus	Trypsin activity begins in N6, reaching the peak in Z2 and decreasing to half 'till PL1.	Galgani *et al.*, 1985.
Penaeus setiferus	Trypsin activity grows in Z1, reaching its maximum in Z3 and decline towards PL1. Until PL140, activity shows little increase. Amylase begins in N5, reaching its peak in M2 and diminishing towards PL1. From PL1 to PL140, activity increases gradually. Diet: microalgae and *Artemia* nauplii.	Lovett and Felder, 1990a.
Penaeus japonicus	Trypsin activity keeps high from Z3 to PL1'with a diet of artificial feed and *Chaetoceros gracilis* (microalga)	Le Vay *et al.*, 1993.
Penaeus japonicus	Trypsin rises in M1, reaching its maximum in M3 and decreasing towards PL1. Amylase reach the peak in Z3. Shrimps were fed on *Artemia* nauplii and *Chaetoceros gracilis* (microalga)	Rodríguez *et al.*, 1994.

Note: N - nauplius, Z - zoea, M - mysis and PL - postlarvae.

Table 2. Changes in energy (J/mg of dry weight) **E**, protein (%) and lipid (%) contents, and C:N ratio for some crustaceans in culture.

Species	E	C:N	Proteins	Lipids	Reference
Macrobrachium malcomsonii (egg to larvae)	decrease				Mathavan *et al.*, 1986
Hyas araneus (megalopa)	decrease	high through the half of the stage and decrease towards ecdysis.	decrease	decrease	Anger, 1987.
Carcinus maenas (zoea I under starvation)	decrease	rises during the four first days and decrease towards ecdysis.			Dawirs, 1987.
Artemia (Instars I to III)	decrease				Leger *et al.*, 1987.
Inachus dorsettensis (zoea to mega-lopa)	decrease after ecdysis and increase in intermolt.	high through the half of the stage, low in the pre and post ecdysis.			Anger, 1988.
Penaeus japonicus (post-larvae 1 to 16)		decrease			Lim and Hirayama, 1993.

Table 3. Relationship between ingestion rate and live food density for some larval shrimps in culture.

Species	Relationship	Reference
Penaeus marginatus	linear	Gopalakrishnan, 1976.
Penaeus indicus	asymptotic	Emmerson, 1980.
Penaeus kerathurus	linear	Yufera *et al.*, 1984.
Palaemonetes varians	asymptotic	Yufera and Rodríguez, 1985a.
Palaemon serratus	asymptotic	Yufera and Rodríguez, 1985b.
Metapenaeus ensis	asymptotic	Chu and Shing, 1986.
Penaeus monodon	asymptotic	Loya-Javellana, 1989.