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The digestive enzymes amylase and trypsin during the development of *Artemia* : effect of food conditions

J. F. Samain, J. Moal, J. Y. Daniel, J. R. Le Coz, and M. Jezequel

Centre Océanologique de Bretagne
B.P. 337, F-29273 Brest Cedex, France

Abstract

Activities of the digestive enzymes amylase and trypsin have been studied during development of *Artemia* (San Francisco, USA) cultured at different concentrations of phytoplankton (*Tetraselmis suecica*).

The synthesis of the two enzymes varies independently throughout the development. Activity levels are controlled by food levels as well as by food composition. To obtain an optimum assimilation yield at a given food level, an adaptive mechanism would involve 1) modulation of ingestion rates based on physicochemical properties of food particles and 2) regulation of synthesis of digestive enzymes by ingestion and chemical composition of the food. Rates of ingestion and assimilation vary more during development than does the yield. Maximum yield is achieved during the exponential growth phase and is related to food requirements. As such, regulation of digestive enzymes during development would optimize the use of the food available, as a function of the food requirements.

Introduction

Many authors have suggested that Crustacea such as *Artemia* (Bellini, 1957ab), the shrimp *Palaemon serratus* (Van Wormhoudt, 1973), and *Penaeus japonicus* (Laubier-Bonichon *et al.*, 1977) show wide variations in protease and amylase activity during the larval development. They described the influence of developmental stages on these variations, as have other authors for mammals (Corring and Aumaitre, 1970 ; Lebas *et al.*, 1971) and Amphibia (Urbani, 1957). Experiments on pre-feeding stages (developing embryos) demonstrated the existence of periods for genetic expression of amylase and protease synthesis (Bellini, 1957ab ; Kulka and Duksin, 1964). Experiments on the dependence of the period of larval development on food, were generally performed with a progressively adapted diet, either natural or modified by the experimenter. It was impossible to conclude to an adaptation of the digestive equipment resulting from the new food, or to a new particular behavior of the animals corresponding to a time or stage dependent expression of the genetic information (Corring and Aumaitre, 1970 ; Lebas *et al.*, 1971). In order to try to solve this question, we have used a uniform diet throughout the experiments reported in this paper; Since the appearance of digestive enzymes is thought to be an indicator of a nutritional requirement (Cuzon, 1970), relationships between ingestion, assimilation, and digestive enzyme synthesis were investigated.

Materials and methods

Dry *Artemia* cysts (San Francisco strain from Metaframe California, USA) were rehydrated in filtered seawater at 22 °C. Nauplii were collected after 24 hr and incubated in 20 l tanks (at the same temperature) under continuous artificial illumination.

Food was provided daily from the second day following hatching at different concentrations and various ratios (k) of algae/*Artemia* protein.

Daily the cultures were cleaned of moults and dead bodies while the seawater medium was regularly replaced and regulated as a function of the k values.

Monospecific cultures of the phytoplanktont *Tetraselmis suecica*, grown by the aquaculture team of the Oceanologic Center of Brittany, were used as food. Batch cultures were started with a single addition of nutrients in 60 l polyethylene bags. The cultures were harvested after 7-10 days at a concentration of 2.10^6 cells/l. Continuous cultures were maintained in 20 l tanks. Each day 1/4 of the culture was removed for use, followed by the addition of a corresponding volume of seawater enriched with nutrients and vitamins (Flassch and Normant, 1974).

The phytoplankton concentration was estimated by microscopic counting in a Malassez cell or electronic counting with a Coulter Counter. Oxygen, pH, and temperature were regularly controlled ; NH_4, NO_2 and the number of bacteria were followed in experiment number 3.

Artemia were sampled at random ; 100 freshly hatched nauplii and three to four adults were necessary for one analysis. The whole organism was ground up in a Thomas blender with distilled water. Digestive enzymes and proteins were analyzed on Technicon autoanalysers using an adapted method (Samain *et al.*, 1977).

The body length was measured under a binocular lens with an ocular micrometer. The stage of development was determined according to the methodology described by Provasoli and D'Agostino (1969).

Estimation of ingestion and assimilation rates by C^{14} were based on a new method (Samain, in preparation) which takes the criticisms expressed by Conover and Francis (1973) into account. C^{14} respiratory- and faecal excretion was measured simultaneously with the apparent C^{14} ingestion rate ; possibilities of recycling of the labeled material were eliminated by an adapted washing procedure. Prior to use, the phytoplankton was uniformly labeled with sodium C^{14}-carbonate.

The first experiment, carried out in duplicate, was performed with a routine phytoplankton culture at a concentration of 300.10^6 cells/l ; $k = 300.10^2/\mu g$. Samples were taken every 2 hr from 8.00 am to 6.00 pm for 25 days after hatching.

In the second experiment three different algal food concentrations were compared (calculated as number of cells per *Artemia* protein weight : $k = 200.10^2/\mu g$, respectively $300.10^2/\mu g$ and $400.10^2/\mu g$) at a constant concentration of 300.10^6 cells/l of routine phytoplankton culture and a duplication of the $300.10^2/\mu g$ conditions. Samples were taken once a day at 10.00 am for 29 days after hatching. Growth rate data were collected during the first twelve days.

The third experiment was carried out with four different phytoplankton concentrations (75, 150, 300, and 600.10^6 cells/l) at a constant k ratio = $600.10^2/\mu g$. Samples were taken once a day at 10.00 am for 24 days after hatching.

The last experiment was performed with 230.10^6 algal cells/l and $k = 330.10^2/\mu g$; the phytoplankton originated from continuous cultures with high nutrient and vitamin concentration. Samples were taken once a day at 10.00 am for 33 days.

For the labeling experiments, part of the *Artemia* population was incubated with phytoplankton uniformly labelled with C^{14}.

Results

Experiment 1

The results (Fig. 1 and 5.1) show that reproducible peaks of amylase and trypsin appear at different times during growth. This phenomenon is distinct from individual and diurnal variations.

Amylase

A major peak appears from the 1st day to the 11th day. It consists of two possible peaks at day 2 and day 6 respectively (L = 0.68 and 1.00 mm). A secondary peak appears from the 12th to the 15th day (L = 2.24 mm). A tertiary peak begining at 23 days (L = 6.3 mm). Levels of specific amylase activity decrease from the first to the third peak (1.1, 0.6, 0.25 units).

Trypsin

A major peak appears from the 1st to the 20th day, consisting of two possible peaks at days 7 and 14 respectively (L = 1.1 and 2.3 mm). A secondary peak appears on the 22nd day (L = 6.3 mm). Levels are 240 units for the first bimodal peak and 60 units for the secondary peak.

Experiment 2

The results (Fig. 2 and 5.2) show that growth of the $k = 300.10^2/\mu g$ duplicates is nearly identical and only slightly inferior to that at $k = 400.10^2/\mu g$; $k = 200.10^2/\mu g$ conditions give a reduced growth.

These various k conditions do not markedly affect the enzyme profiles except for a distinctly lower amylase level at $k = 400.10^2/\mu g$ conditions and lower trypsin level under the $k = 200.10^2/\mu g$ conditions.

Amylase

A major bimodal peak occurs from the 1st to the 7th day (L = 0.6 to 1.0 mm), a secondary peak occurs from the 7th to the 12th (L = 1.0-1.4 mm), a tertiary one from the 19th to more than 29 days (L > 3.2 mm). Highest levels of specific amylase activity are 0.75-0.47-0.70 units. With the $k = 400.10^2/\mu g$ conditions, lowest levels are 0.5-0.20 units.

Trypsin

A continuous low level (maximum : 80) activity decreases from the 1st day to the 24th. This broad and low peak is perhaps bimodal : 1-13 days (L = 0.6-2.0 mm) and extends beyond the 19th day (L > 5.0 mm). The levels of these two possible peaks are similar (70 units). In comparison with the first experiment, the mean activity of amylase and trypsin is generally

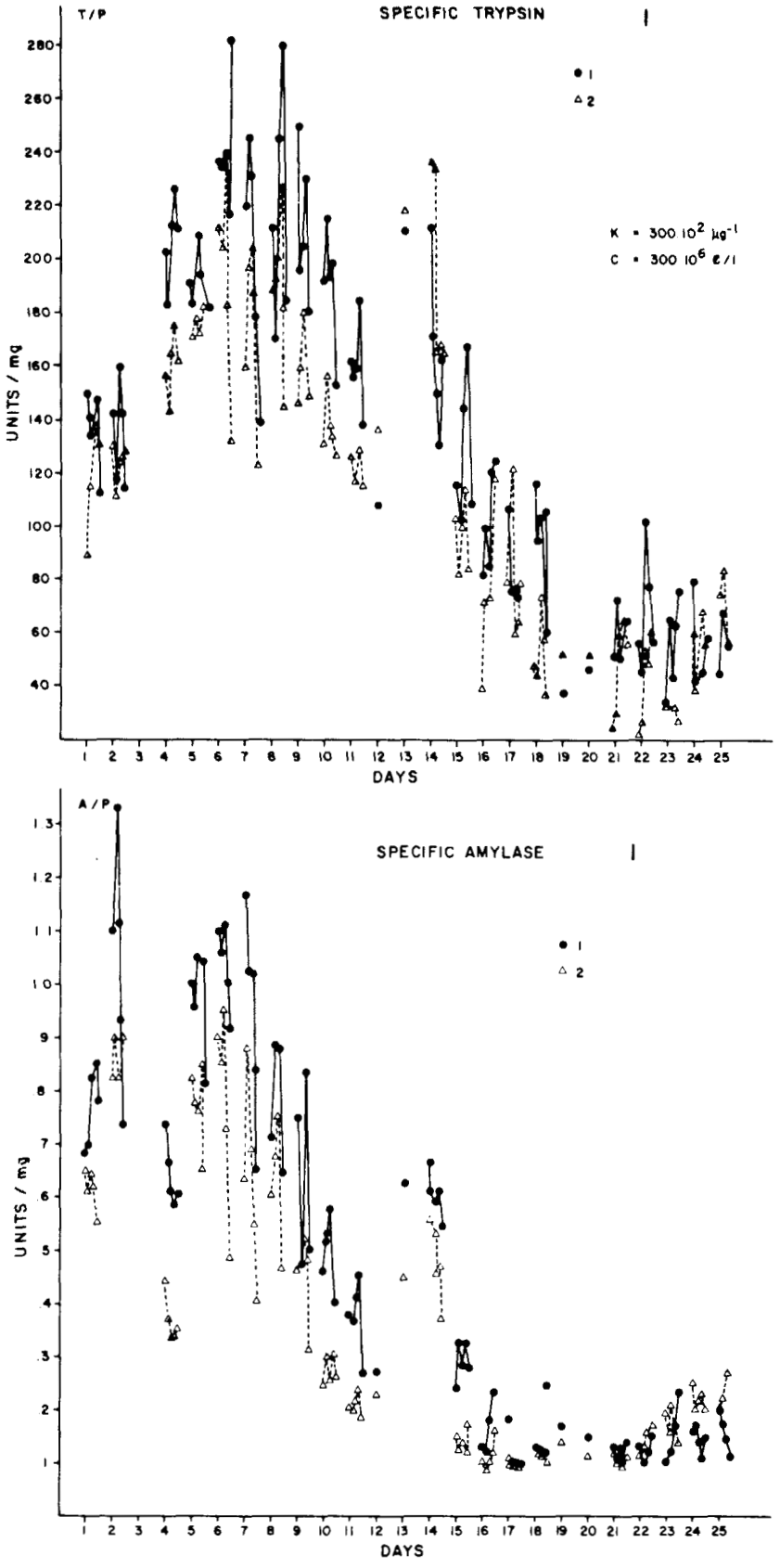

FIG. 1. Experiment 1. Specific trypsin and specific amylase activity during the first 25 days after hatching. Experiment carried out in duplicate. Phytoplankton concentration $C = 300.10^6$ cells/l ; $k = 300.10^2/\mu g$.

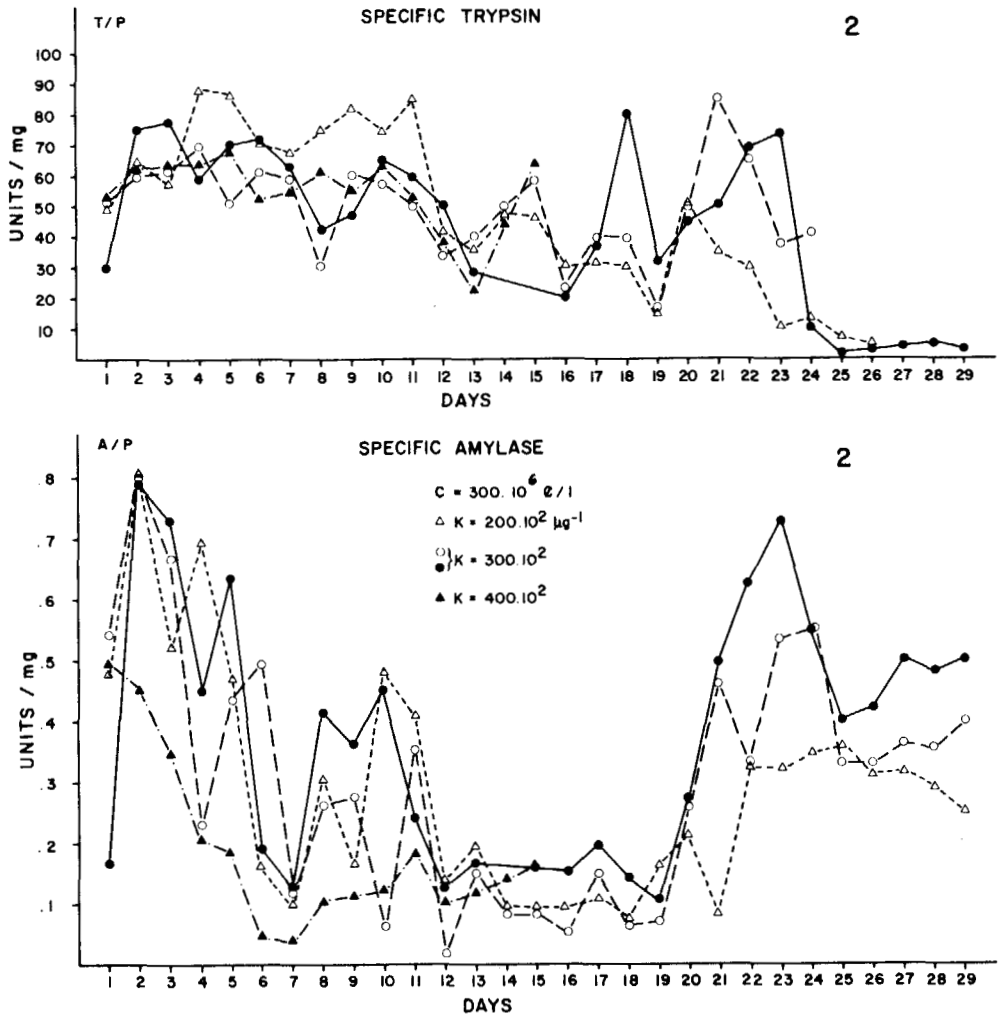

FIG. 2. Experiment 2. Specific trypsin and specific amylase activity during the first 23 days after hatching. Phytoplankton concentration : $C = 300.10^6$ cells/1 ; k variable $\Delta k = 200.10^2/\mu g$; ○ ● $k = 300.10^2/\mu g$; ▲ $k = 400.10^2/\mu g$.

lower in this experiment (amylase maximum = 0.70 unit/1.1 unit ; trypsin maximum : 70/260 units). The $k = 200.10^2/\mu g$ conditions show a broad bimodal peak reaching 80 units and a lower secondary peak of 50 units.

Observations on developmental stages show that the trypsin peak occurs at the metanauplius stage and decreases slowly until the appearance of the first adults where trypsin levels increase again. The first amylase peak appears at the metanauplius stage, decreases rapidly, while the second one coïncides with a majority of the population at stage V. Subsequently, the levels are depressed from stage VI to X, and the third peak appears as the first animals reach the adult stage.

EXPERIMENT 3

The results (Fig. 3 and 5.3) show that growth and enzyme levels are identical at these four conditions. However, growth is higher than in experiment 1 or 2.

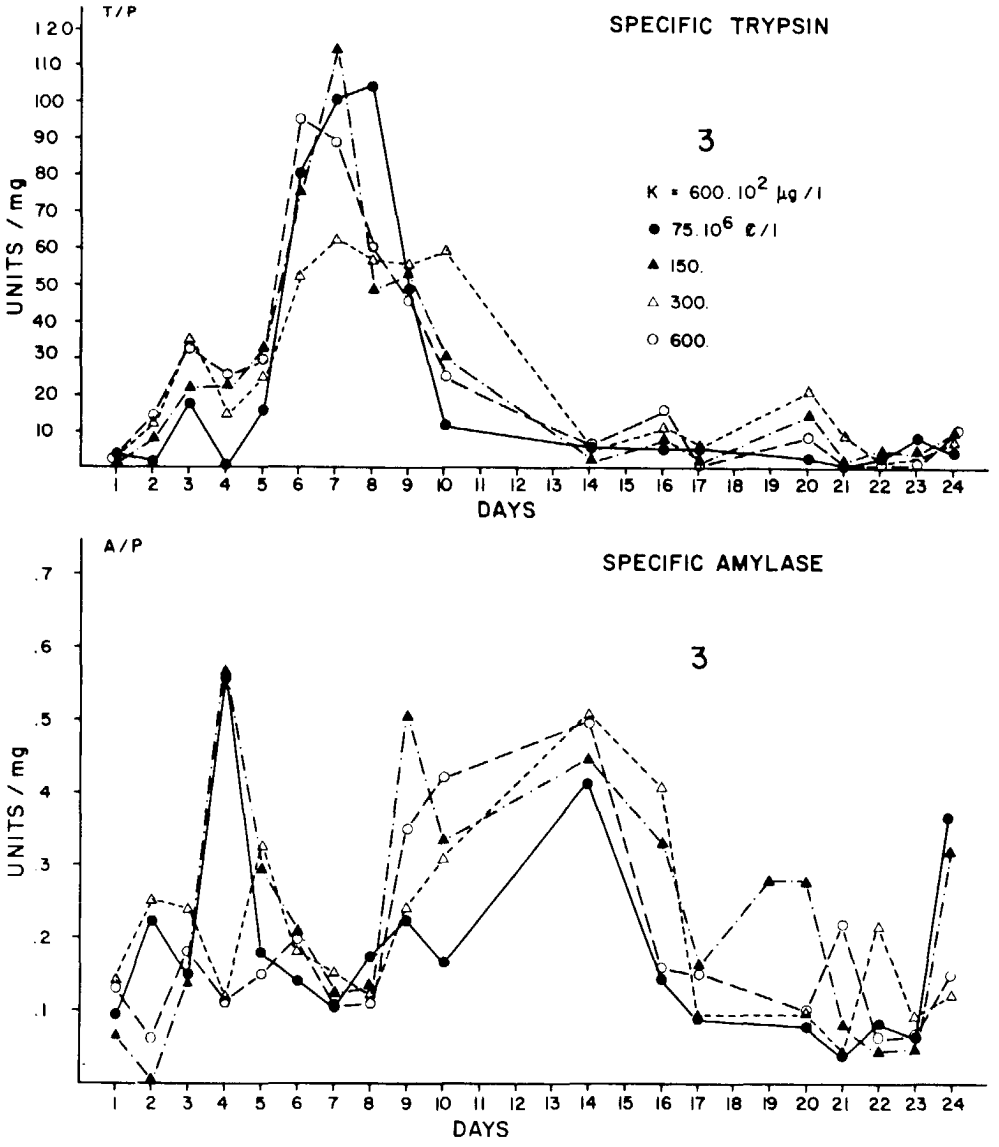

FIG. 3. Experiment 3. Specific trypsin and specific amylase activity during the first 24 days after hatching. Phytoplankton concentrations : ● C = 75.10⁶ cells/l ; ▲C = 150.10⁶ cells/l ; △C = 300.10⁶ cells/l ; ○ C = 600.10⁶ cells/l ; k constant = 600.10²/µg.

Amylase

Two main peaks of amylase occur during the first week (L = 0.60-2.80 mm) and from the 8th to the 17th day (L = 3.5 to 8.9 mm) highest levels are 0.57 and 0.50 units. The first peak is possibly bimodal (1-3, 3-7 days ; L = 0.6-1, 1-2.8 mm).

Trypsin

A sharp peak of trypsin (maximum value = 110 units) is visible between 4 and 10 days (L = 1.4 to 4.5 mm) preceeded by a small peak on the 3rd day (L = 1.1 mm). After this, the activity is at a very low level. Mean activities of these two enzyme levels are lower than in the preceeding experiments.

Experiment 4

The fourth experiment was performed with *Tetraselmis* from continuous cultures having higher concentrations of nutrients than in the preceeding experiments. These conditions induce low C/N and carbohydrates/protein ratios in algae with higher protein and lower carbohydrate contents (Moal *et al.*, 1978).

The results (Fig. 4 and 5.4) show that growth is nearly the same as in experiment 1.

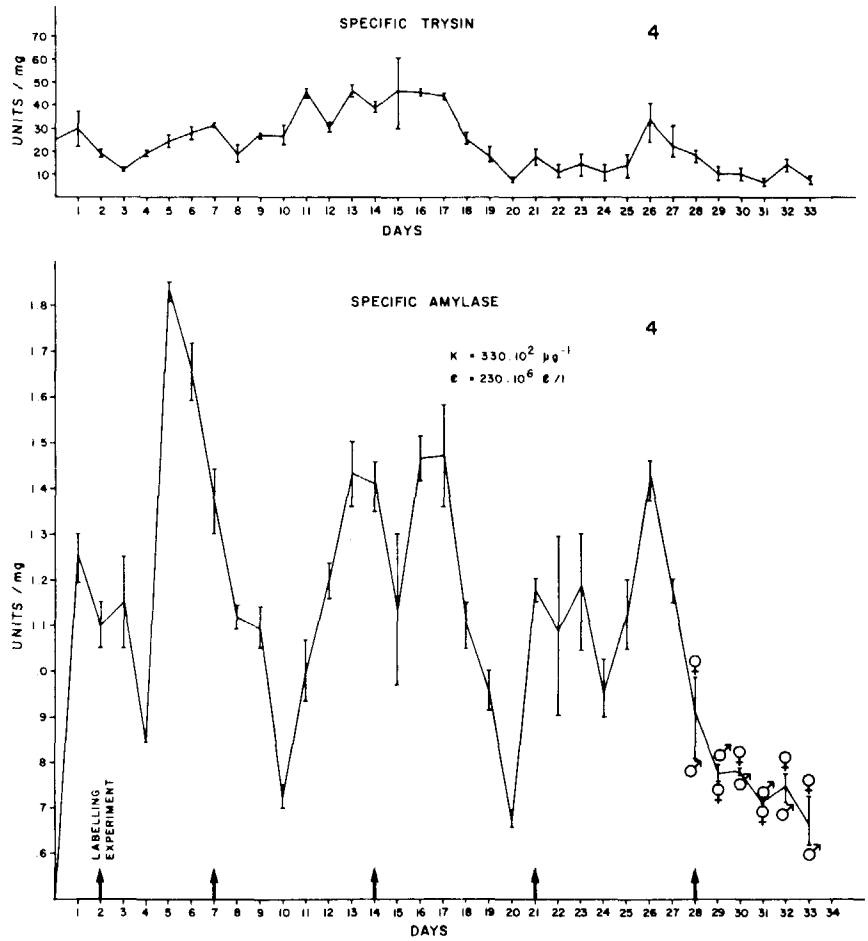

Fig. 4. Experiment 4. Specific trypsin and specific amylase activity during the first 26 days after hatching. Phytoplankton concentration : $C = 230.10^6$ cells/1 ; $k = 330.10^2/\mu g$. Experiment carried out in duplicate. Males (♂) and females (♀) are separated when sexual dimorphism is visible. Labeling is performed at five different moments during the development.

Amylase

Three bimodal peaks of equal magnitude occur during growth, the first one appearing between days 1 and 10 with a maximum to 1.25 and 1.65 units (L = 0.6 to 1.0 mm). The second peak appears between 10 to 20 days, with a maximum at 1.4 and 1.45 units (L = 2 to 4 mm), while the third occurs between 20-28 days, with maximum values at 1.2 and 1.4 units (L = 6.3 to 9.0 mm). These maximum values are higher than in the preceeding experiments.

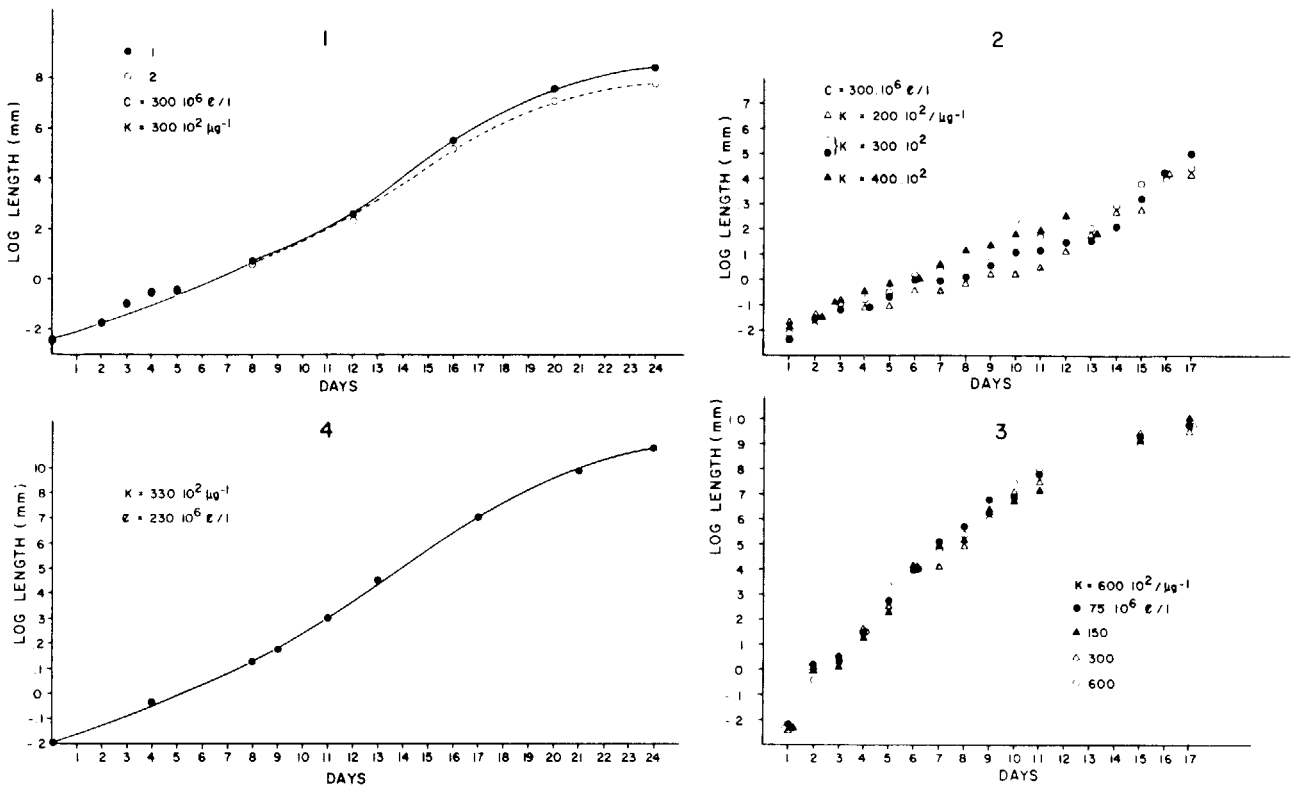

FIG. 5. Log length (mm) in function of time in four experimental conditions.

5.1. $k = 300.10^2/\mu g$; $C = 300.10^6$ cells/l.
5.2. $k_1 = 200.10^2/\mu g$; $k_2 = 300.10^2/\mu g$; $k_3 = 400.10^2/\mu g$; $C = 300.10^6$ cells/l.
5.3. $k = 600.10^2/\mu g$; $C_1 = 75.10^6$ cells/l ;
$C_2 = 150.10^6$ cells/l ; $C_3 = 300.10^6$ cells/l ; $C_4 = 600.10^6$ cells/l.
5.4. $k = 300.10^2/\mu g$; $C = 230.10^6$ cells/l.

Trypsin

A major peak occurs between 10 and 20 days (L = 1.4 to 5.0 mm) with a maximum at 45 units. It is preceeded by 30-32 unit bimodal peaks, one occurring on the 1st day (L = 0.63 mm) and one on the 7th day ($L \simeq 1$ mm). A sharp peak of 32 units occurs between 25-29 days ($L \simeq 9$ mm). These maximum values are lower than in the preceeding experiments.

Occurrence of amylase and trypsin peaks as a function of time or length of the animals are summarized in Fig. 6. Three main peaks of amylase and two main peaks of trypsin generally occur during the development, except in experiment 3, where growth has been considerably higher.

Amylase

Considering the time scale in the three other experiments, the first peak occurs between the 1st and the 10th day, the second between the 8th and the 20th, and the third after the 20th day. On the length scale, the first occurs between 0.6 to 0.9 mm, the second one between 1 to 6 mm, the third one between 3 to 9.5 mm.

Trypsin

Again, on the time scale, the first peak occurs between 1-20 days, and more usually between 1-12 days, while the second is after 19-20 days. In terms of length, the first peak occurs between 0.6-5.0 mm and more usually between 0.6-2.0 mm.

A scheme summarizing the four experiments is shown in Fig. 6.

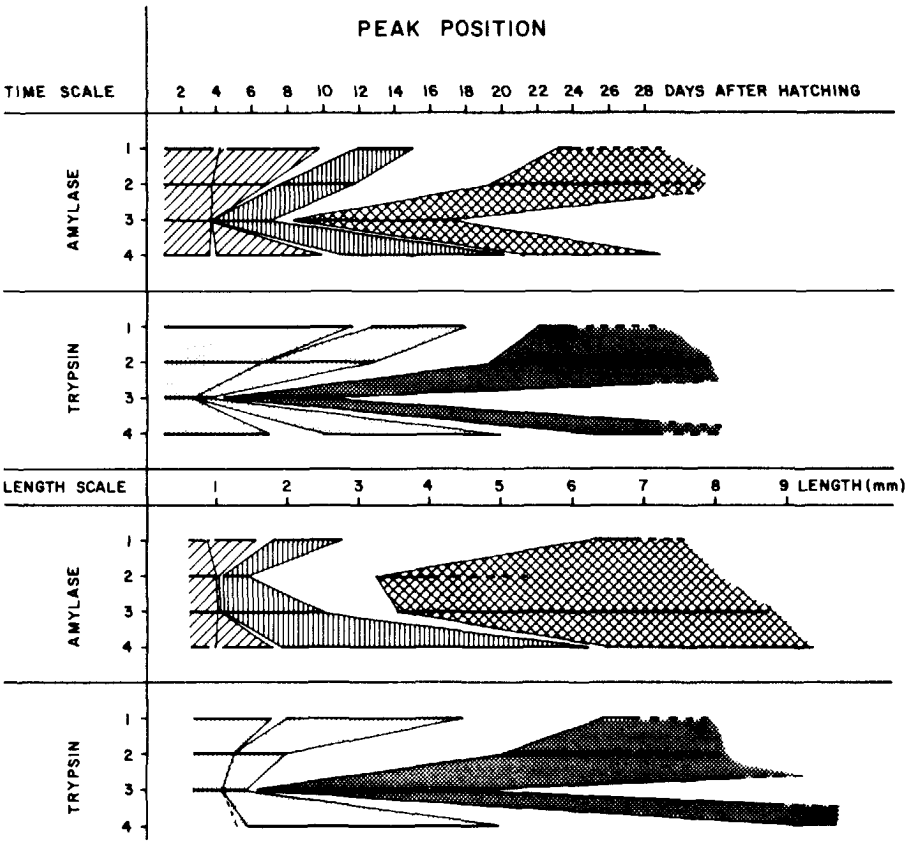

FIG. 6. Summarizing scheme of the four experiments: peak positions of amylase and trypsin specific activity during the development of *Artemia* in function of time (days after hatching) or body length (mm).

Discussion

Very distinct variations of amylase and trypsin activity are observed during the development of *Artemia*. This phenomenon is independent from diurnal and individual variations.

REGULATION BY GROWTH

When comparing the four experiments, it is obvious that the high growth rate in experiment 3 has restricted this phenomenon to a reduced time scale. On the other hand a low rate of growth spreads the phenomena along the time scale (experiment 4). Two major groups of amylase and trypsin peaks are discernable. The first group is apparent during the early

stages of development, and the second at the beginning of adult stages. Amylase peaks are more distinct from one another than trypsin peaks. Thus, three possible amylase peaks are visible in the first group, a bimodal peak and a more distinct single peak. A possibly bimodal trypsin peak is suggested in the first group (experiments 1, 2, 4). The second group seems to be quite homogenous. In the third experiment, the second broad amylase peak relates to the second group peak in experiment 1, 2, and 4 and so does the major trypsin peak of this experiment. Therefore the first discernable group of amylase peaks could be a surimposition of the three ones usually occuring first, and the little first trypsin peak would contain the first bimodal one. The peculiar spectrum of digestive enzymes observed in experiment 3 clearly demonstrates that time is not a good criterion for peak occurrence.

On the length scale, some similarities appear between the different spectra. Other authors have suggested that this peak occurence is correlated with developmental stages (Corring and Aumaitre, 1970 ; Lebas *et al.*, 1971 ; Van Wormhoudt, 1973 ; Laubier-Bonichon *et al.*, 1977). Our observations tend to corroborate this hypothesis. High digestive enzyme activity is visible during nauplius and metanauplius (until stage V) and again at the preadult stages. Nevertheless, comparing the relative position of the amylase-trypsin peaks, the second group of trypsin peaks sometimes coincides with the second amylase group (experiment 1) (preadult stage), or with an amylase depression (stages VI-X) (experiment 3) or between these two positions (experiment 2 and 4). As the relative peak positions fluctuate, it is apparent that stages are not a rigourous explanation for peak occurrence.

These results show that enhancement of trypsin and amylase synthesis are independent. Howard and Yudkin (1963) found the same result for the rat pancreas. Nevertheless, the occurence of these enzymes is not strictly correlated to a length – or time scale, but to a general scheme varying with growth.

Regulation by chemistry of the food

The chemical composition of the diet influences the make-up of digestive enzymes as has been demonstrated for mammals by Pavlov (Vasilev, 1893 ; Jablonsky, 1895), Grossman *et al.* (1942), Desnuelle and colleagues (Reboud *et al.*, 1962, 1964, 1966 ; Ben Abdeljlil *et al.*, 1963 ; Ben Abdeljlil and Desnuelle, 1964 ; Marchis-Mouren *et al.*, 1963), Corring and Aumaitre (1970), O. Mack *et al.* (1975), and Bucko *et al.* (1976). The same has been suggested for insects by Shambaugh (1954), Langley (1966), Engelmann (1969), Yang and Davies (1968), Hosbach *et al.* (1972) and for Crustacea by Van Weel (1960). In each experiment reported here, *Artemia* were fed a single species of food, therefore the occurrence of peaks should be independent of the nature of the food. The nature of food can be characterized by different species of phytoplankton or particles, but also by the chemical composition of the food. Chemical composition of *Tetraselmis* cultures had been systematically monitored and it was demonstrated that the chemical composition of these algae was highly influenced by nutrients and stages of the culture (Moal *et al.*, 1978). Nevertheless no correlation was found with peak occurrence. The intensity of peaks is so variable that they sometimes disappear (first bimodal peak of amylase experiment 3, second amylase peak of k $400.10^2/\mu g$ in experiment 2, first mode of the trypsin bimodal peak in experiment 4). The influence of chemical composition of the food on the intensity of enzyme synthesis can be extrapolated from the present study. The intensity of digestive enzyme systems varies more from one experiment to another than with

concentrations or k ratio variations within an experiment. Experiments 1, 2, and 3 have indeed been performed with cultures from uncontrolled batch production (high and varying C/N and carbohydrates/protein ratio), whereas experiment 4 was carried out with controlled continuous cultures under conditions of constant saturated nutrient conditions (low C/N and carbohydrates/proteins ratio). The larger differences in the two enzyme levels observed between experiments with batch cultured algae and continuously cultured algae, corroborate the hypothesis that digestive enzyme levels are related to fluctuations in the chemical composition of the food.

Regulation by ingestion

In a previous paper (Samain *et al.*, 1975), we have demonstrated a relationship between various concentrations (0, 35, 75, 150, 300.10^6 cells/l) of the same food, and amylase and protease levels of *Artemia* of different length categories, as well as a relationship between ingestion and digestive enzyme levels. Correlations of digestive enzyme levels with ingestion processes and chemical composition of the diet have been observed for zooplankton organisms (Boucher *et al.*, 1975 ; Mayzaud and Poulet, 1978). Last but not least a short starvation of previously fed *Artemia* results in a *de novo* peak of specific amylase and trypsin which we attribute to the sudden lack of food (Fig. 7) (Samain, in preparation). These results are in accordance with a general hypothesis of the regulation of digestive enzyme level by ingestion (Langley, 1966).

Indeed a changed chemical composition of the phytoplankton can correspond with physical modifications of the cells influencing in turn the grazing activity and consequently the ingestion processes. We have noticed that the general appearance of *Tetraselmis* cells varies with the cultivation conditions : clustered cells are observed in stationary phases, sedimentation occurs at high pH values and mobility is lower at higher nutrient levels. Conover (1966) found that the ingestion rate of copepods was markedly depressed in old cultures of algae. Regulation of digestive enzymes by the chemical composition of particles could be the result of the effect of ingestion modifications.

Regulation by food requirements

In the four experiments reported above only the k ratio significantly influences the growth of *Artemia* and seems to be the limiting factor during the entire study. When k is constant, modification of algal concentrations probably induces few variations in ingestion and, as a consequence in digestive enzyme levels (experiment 3). When k varies, some differences can be observed (experiment 2) at a constant high concentration ($c = 300.10^6$ cells/l). Resultant growths are quite modified and some differences in enzyme levels are significant ($k = 400.10^2$ amylase, $k = 200.10^2$ trypsin (experiment 2), $k = 600.10^2/\mu g$ (experiment 3)). During his study on the influence of quantity and concentration of food on *Artemia* growth, Mason (1963) found the same phenomena. The k factor corresponds to the food available. We have to compare it to the food requirement. For an identical and limiting k (experiments 1, 2, 4) the results in terms of growth are quite the same. As digestive enzyme levels are very different from one experiment to another, it is possible that an adaptive mechanism is operating by way of digestive enzyme synthesis to modulate the assimilation efficiency in relation to the varied ingestion levels (Sushchenya, 1970). This adaptive mechanism would consist of the

modulation of ingestion rates induced by the physicochemistry of particles (Provasoli and d'Agostino, 1969 ; Poulet and Marsot, 1978) in the regulation of digestive enzyme synthesis, as a function of these ingestion levels, and as a function of the chemical composition of particles, to obtain an optimum assimilation yield in relation to food requirement.

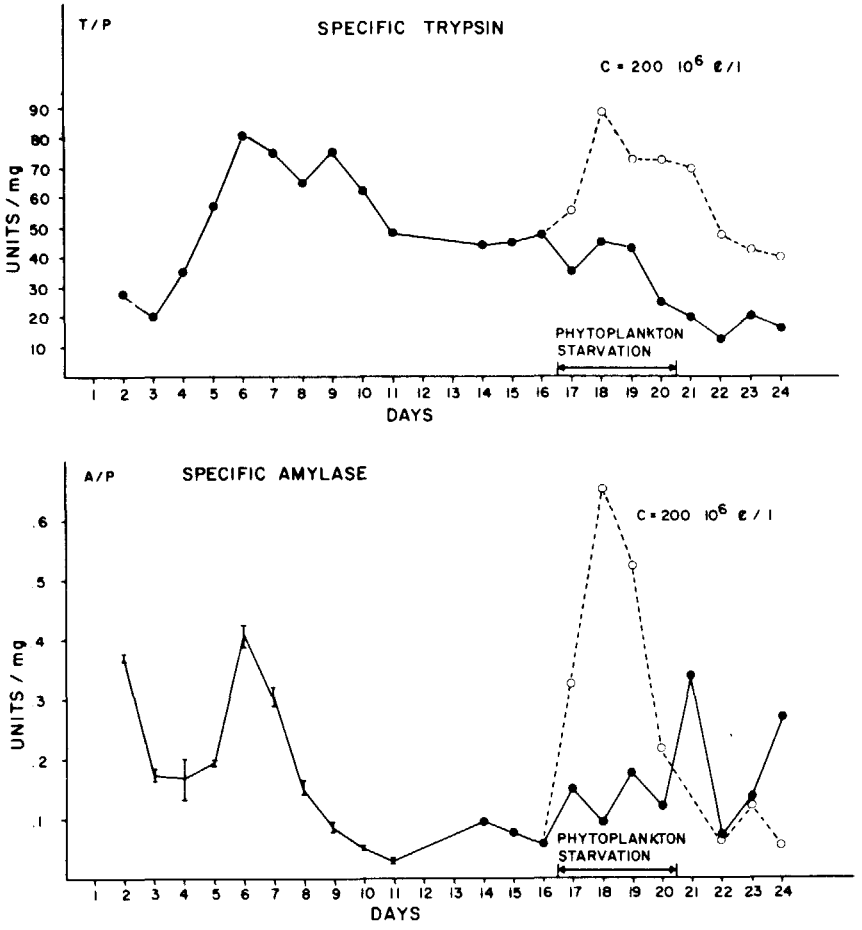

FIG. 7. Experiment on short term starvation during *Artemia* development. Effect on 4 days starvation on specific amylase and trypsin activity of *Artemia* usually fed phytoplankton at a concentration of 200.10^6 cells/l.

Many authors suggested that the variations of digestive enzymes during developmental processes can be attributed to food requirements (Van Wormhoudt, 1973 ; Laubier-Bonichon *et al.*, 1977). Nutritionists take into account observations of the digestive equipment for the determination of food requirements (Cuzon, 1970). Some observations on varying growth efficiency at different developmental stages have already been reported for *Artemia* (Mason, 1963 ; Reeve, 1963) and copepods (Mullin and Brooks, 1967 ; Päffenhöfer, 1976) ; varying ingestion levels have also been mentioned (Marshall and Orr, 1956 ; Paffenhöfer, 1971). The results are in accordance with the hypothesis suggested above.

Our fourth experiment has been performed with five determinations of ingestion and assimilation rate during growth by a new C^{14} method (Samain, in preparation). Results show

that digestive enzymes vary with assimilation and ingestion in a well defined pattern which can be mathematically expressed as follows (Samain *et al.*, in preparation):

$$\frac{\text{Ass}}{\text{k(E)}} = \frac{\text{Ass max(I)}}{\text{K} + \text{(I)}}$$

where Ass = Assimilation rate
Ass max = maximum assimilation rate
I = ingestion rate
E = digestive enzyme specific activity
k = proportional constant
K = ingestion rate corresponding to $\frac{\text{Ass max}}{2}$

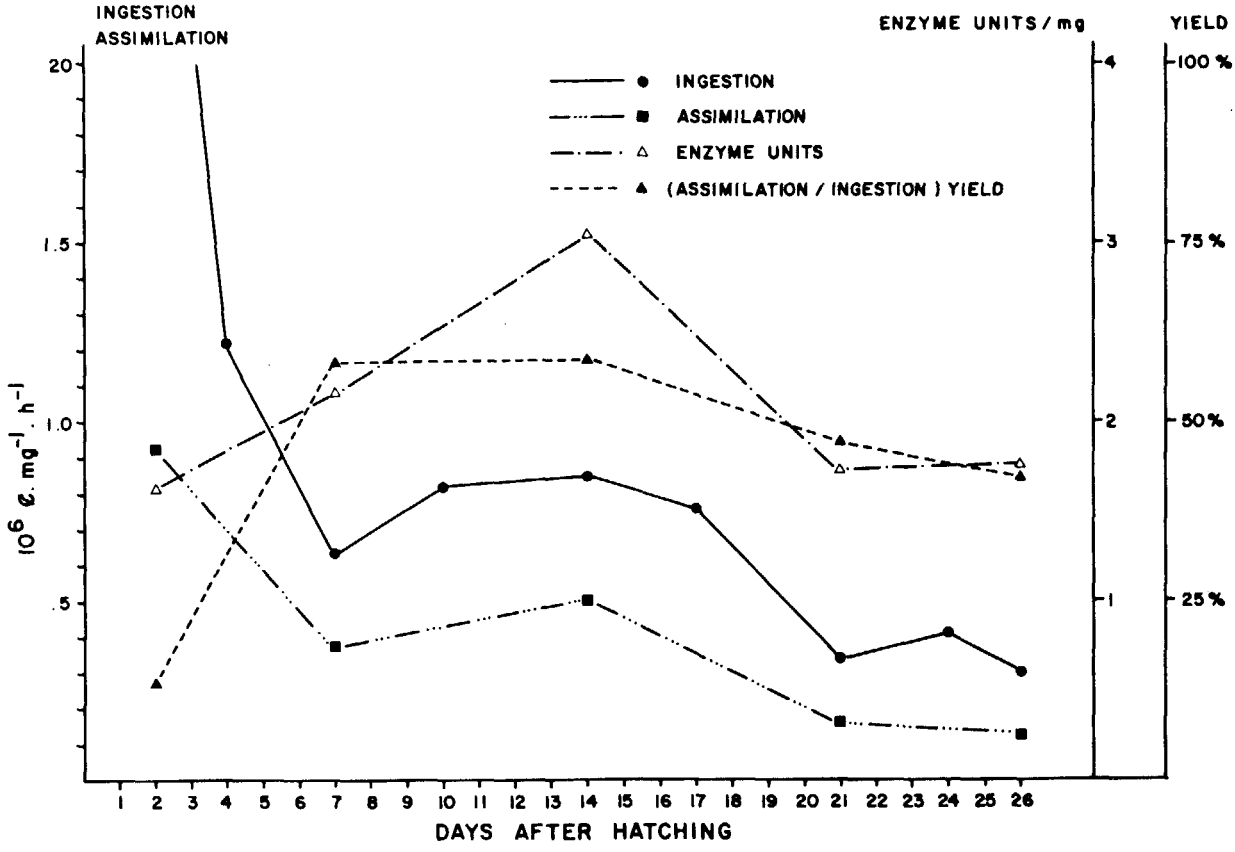

FIG. 8. Experiment 4. Ingestion and assimilation rates, enzyme units = total (amylase + 0.045 trypsin) specific units ; mean value m of 18 daily samples, s = 0.1 m (assimilation/ingestion) × 100 yield, in function of time.

Furthermore ingestion and assimilation rates vary during the growth when food parameters (concentration and k ratio) are constant (Fig. 8). These latter results demonstrate the existence of an adaptive mechanism involving ingestion rates and digestive enzyme synthesis. They corroborate the hypothesis of specific periods of nutritional requirements in relation to

development. In the fourth experiment, two periods of higher assimilation are visible at the first stages of development (nauplius, metanauplius) and at the preadult stages, with an optimum of the assimilation/ingestion yield during the exponential phase and a lower yield during an apparent latent and stationnary phase. The occurrence of a latent phase depends on the experiment, and could appear when the assimilation yield is low, or when ingestion and enzyme-stage dependent syntheses are not in good accordance with food characteristics. The specific activity of amylase and trypsin has been summed in the mathematical expression. From all the experiments presented here, it appears that the relative amylase/trypsin ratios vary from one expriment to the other. This could point to mechanisms of optimisation in the use of food components.

As moulting or developmental phenomena are the result of complex interactions between genetic expression, hormonal regulation, and ecological conditions such as food, temperature, light, *etc.* (Fisk and Shambaugh, 1952 ; Van Weel, 1960 ; Langley 1966 ; Rutter *et al.*, 1968 ; Engelmann, 1969 ; Dickard Felber, 1974 ; Adler and Kern, 1975 ; Butcher, 1975 ; Felber *et al.*, 1975 ; Wojcik *et al.*, 1975 ; Rudich and Butcher, 1976), it is obvious that no direct relationship can be established between stages and genetic expression as translated by enzyme synthesis. Such a relation is regulated by all these phenomena occurring, from the possibility of genetic expression to the final result. As such, the occurrence of digestive enzyme activity is probably the result of the genetic expression during development processes (as was demonstrated by Bellini (1957ab) during the embryonic development) but this expression is highly modulated by ecological factors (Rutter *et al.*, 1968) influencing the developmental phenomena, and in particular by the good relationship between food parameters and nutritional requirements.

Summary

The digestive enzymes amylase and trypsin in *Artemia* vary during growth according to a general pattern. Three distinct peaks of amylase (the first with a double mode) generally occur from the nauplius stage to the adult. For trypsin there are two major peaks with a possible double mode on the first one ; the trypsin peaks are broader than the amylase peaks.

A genetic regulation, in relation to nutritional requirements seems to be at their origin (genotypic regulation), but their occurrence varies with food conditions (phenotypic regulation).

The intensity of the activity of these digestive enzymes varies strongly when the food, at high concentrations, undergoes physicochemical changes.

Assimilation, resulting from digestive enzyme activity on ingested food, depends on developmental stages. In our experimental conditions, two periods of higher activity are visible : metanauplius stage and preadults stage ; assimilation/ingestion yield is optimum during the exponential phase. These results corroborate the hypothesis of specific periods for food requirement during the development.

The two digestive enzymes studied are synthesized independently. Their respective intensity is dependent on the food characteristics and seems to result from an adaptive mechanism to particular requirements of *Artemia* at every stage. These results confirm that *Artemia* is very adaptable with respect to food requirements.

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