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## Possible processes of nutritive adaptations for zooplankton: a demonstration on *Artemia*

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### Summary

The ingestion rate and the resulting assimilation calculated by means of a law taking into account activities of the digestive enzymes (amylase and trypsin) on the carbohydrates and proteins ingested, are determined in two experiments on *Artemia*. They are fed the same species of phytoplankton (*Tetraselmis suecica*), at the same concentration but with two chemical compositions. In one experiment, the cells are rich in carbohydrates and poor in proteins, and in the second one they are poor in carbohydrates, rich in proteins. Different observed ingestion rates induce a balance in proteins, and a large difference in the carbohydrates ingested. When digestive enzymes are taken into account, the assimilated carbohydrates and proteins are similar (explaining the similitude of the growth rate observed). The assimilation yield study shows that digestive enzymes induce a better digestion of chemical compounds in low concentrations in the food. That could correspond to a regulation of assimilation as a function of requirements of *Artemia*. Requirements would be the first internal factor that regulates the nutritional behavior. So two processes are possible for *Artemia* to obtain the sufficient quantity of food: regulation of ingestion rate, probably depending also on olfactory mechanisms, and regulation of assimilation by the way of digestive enzymes. The importance of these processes is discussed as a function of environmental conditions.

### Introduction

The mechanisms of food ingestion have been widely studied in order to estimate production of zooplankton. There is considerable evidence of relations between feeding rates and concentrations of particles and of various levels of saturation (ADAMS and STEELE 1966, PAFFENHÖFER 1971, FROST 1972, 1975, MULLIN and STEWART 1975, LEHMAN 1976, MAYZAUD and POULET 1978), between feeding rate and chemical composition (PROVASOLI and D'AGOSTINO 1969, POULET 1976). The effects of the particle size have been demonstrated (POULET 1973, 1974, 1977, 1978), as also olfactive selectivity (FRIEDMAN and STRICKLER 1975, POULET and MARSOT 1978). Calorific content is considered to have no effect (FUJI 1962, LEIGHTON and BOOLOOTIAN 1963, LEIGHTON 1966, PAINE and VADAS 1969, CAREFOOT 1973). The second step necessary to estimate production, that is the ration utilization, seems to be difficult to deduce from ingestion (McMAHON and RIGLER 1965, BRETT 1971, CALOW 1975, HARRIS and PAFFENHÖFER 1976, PECHENIK and FISHER 1979). Apparent contradictory results show that another variable was not taken into account for assessment of the relation between ingestion and assimilation. This variable could be the activities of digestive enzymes and their catalytic efficiency.

We have studied the effect of the chemical composition of the food on the two possible steps of regulation of assimilation, i.e. ingestion and digestion. The grazing rates and the activities of the digestive enzymes (amylase and trypsin) have been recorded throughout growth in an experiment using *Artemia* fed with phytoplankton of the same species (*Tetraselmis suecica*) chemically modified by a nutrient effect to obtain two different compositions (MOAL et al. 1978).

In this paper, we have attempted to evaluate the effect of digestive enzyme activity on the ingested carbohydrates and proteins observed.

### Material and methods

Phytoplankton: three different nutrient media were used in the culture of the phytoplankton (see MOAL et al. 1978): high nutrient and low nutrient cultivated cells; an intermediary medium was also used, but the chemical composition was similar to that obtained with the low nutrient medium.

**Table 1**

Mean chemical composition of the food *Tetraselmis suecica*.  
 $\mu\text{g}/10^6\text{C}$ , n = 14, s = standard deviation.

Nutrient Concentration	<i>Tetraselmis</i> Carbohydrates		<i>Tetraselmis</i> Proteins		<i>Artemia</i> Experiment
	m	s	m	s	
Low	71	25	29	6	1
Medium	65	21	29	5	2
High	22	3	54	6	3

*Artemia* cultures: general conditions for culture have been reported in a preceding paper (SAMAIN et al. 1980). *Artemia* (San Francisco Bay, California, USA) are cultured from the nauplii to the adult stages in 20 l tanks at 22° C under continuous artificial illumination. Daily the cultures were cleaned and the phytoplankton provided at a constant concentration ( $150 \cdot 10^3\text{C l}^{-1}$ ) and a constant (k) ratio of algae / *Artemia* protein ( $k > 500 \cdot 10^3\text{C}/\text{Artemia protein } \mu\text{g}$ ). Three experiments were performed: experiment 1 and 2 with cells cultured in poor and medium nutrients conditions and experiment 3 with cells from high nutrient medium. Every day grazing rates were determined, and the chemical composition of the phytoplankton cultures was assessed (C/N, carbohydrates, proteins, chlorophyll, phaeopigments) (MOAL, in prep.). *Artemia* were sampled and length, weight, proteins, amylase and trypsin were determined (SAMAIN et al. 1977).

Ingestion: In the present study, I is calculated every day from the grazing data expressed in terms of carbohydrates or proteins and taking into account daily measurement of chemical composition of the ingested cells.

Assimilation was calculated from a law taking the ingestion and the activities of digestive enzymes into account (SAMAIN et al. 1980):

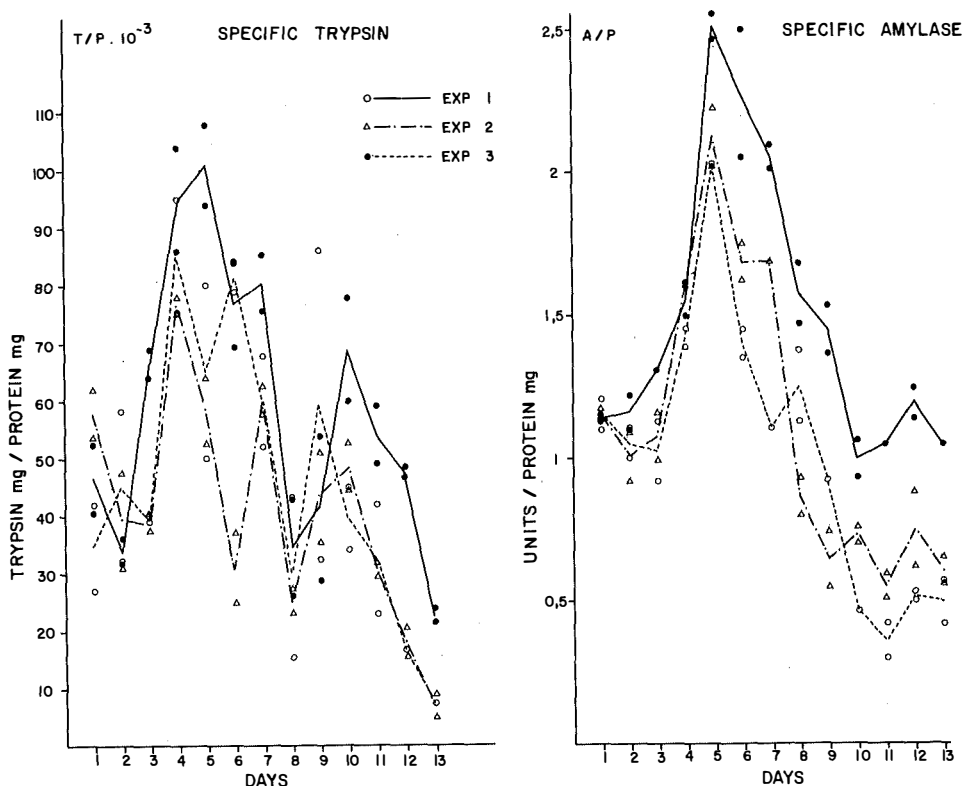
$$\text{Ass} = \frac{\text{Ass max } k (E) (I)}{K + I}$$

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

$$\frac{\text{Ass max}}{2}$$

This law has been established from studies on *Artemia* cultivated from nauplii to adult stages under the same conditions. They were fed with the same phytoplankton (*Tetraselmis suecica*). Assimilation rate and ingestion rate were determined by appropriate  $^{14}\text{C}$  methodology. The constants Ass max, k, K were established for each enzyme (amylase, trypsin) respectively with algae ingestion values expressed in carbohydrates and proteins.

The assimilation yield is the ratio Ass/I.



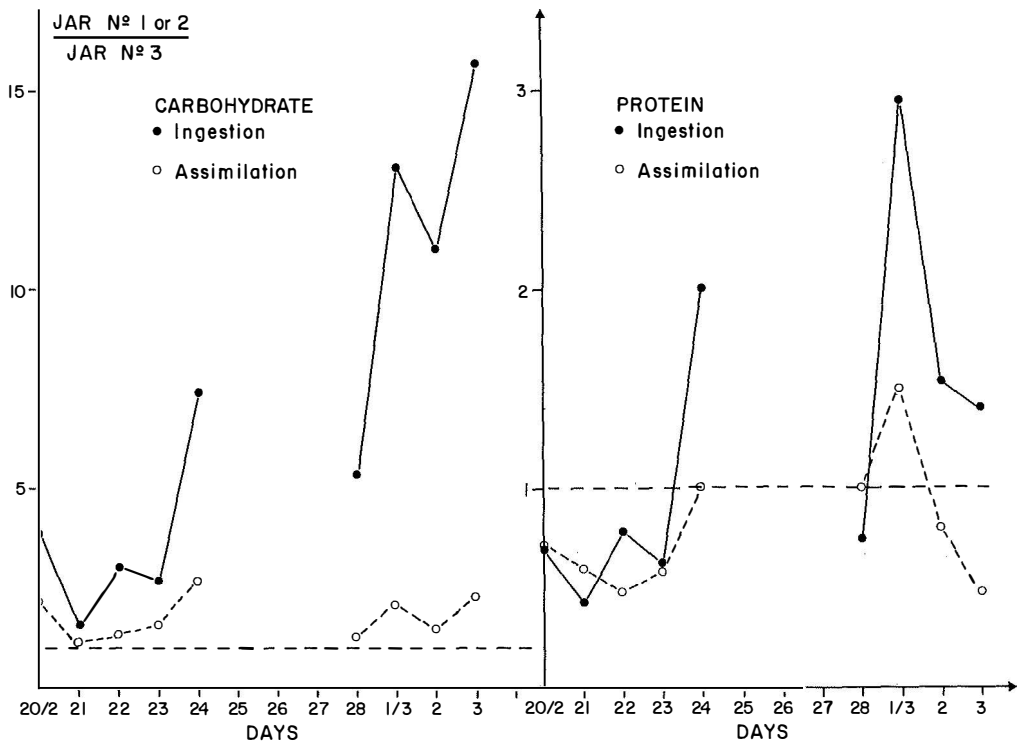
**Figure 1**

Specific trypsin and specific amylase activity during the first to 13th day after hatching. Phytoplankton concentration:  $C = 150 \cdot 10^6 \text{ } \ell^{-1}$ ;  $k > 500 \cdot 10^2 \text{ } \ell / \text{Artemia protein } \mu\text{g}$ . Sampling in duplicate

## Results

The adaptative mechanism: Chemical composition of algae from low and medium nutrient concentrations was not significantly different. Ingestion rate and digestive enzymes levels observed from corresponding *Artemia* experiments (exp. 1 and 2) were also comparable (Fig. 1). So we consider these experiments as duplicates, corresponding to *Artemia* fed on cells with high carbohydrate and low protein content. We compared this experiment (1–2) to experiment 3 where food chemistry (high proteins low carbohydrate contents) and ingestion rate were different: the ratio  $R_I = \text{Ingestion exp 1–2} / \text{Ingestion exp 3}$  was studied in order to compare the grazing between exp 1–2 and exp 3; and the ratio  $R_{\text{ass}} = \text{Assimilation exp 1–2} / \text{Assimilation exp 3}$  was examined in order to compare the assimilated food between exp 1–2 and exp 3 (Fig. 2).

1. Carbohydrates: The two diets led to an increasing difference in the carbohydrates ingested during growth, up to 15 times more in exp 1 and 2 than in exp 3. This difference is negligible when amylase is taken into account: Assimilated carbohydrates are never much more than twice as much in exp 1–2 than in exp 3.
2. Proteins: The observed difference of ingestion values between the two diets are less important than for carbohydrates. The largest observed difference is in ingested protein levels at the end of the growth period (> double on two data). The difference in



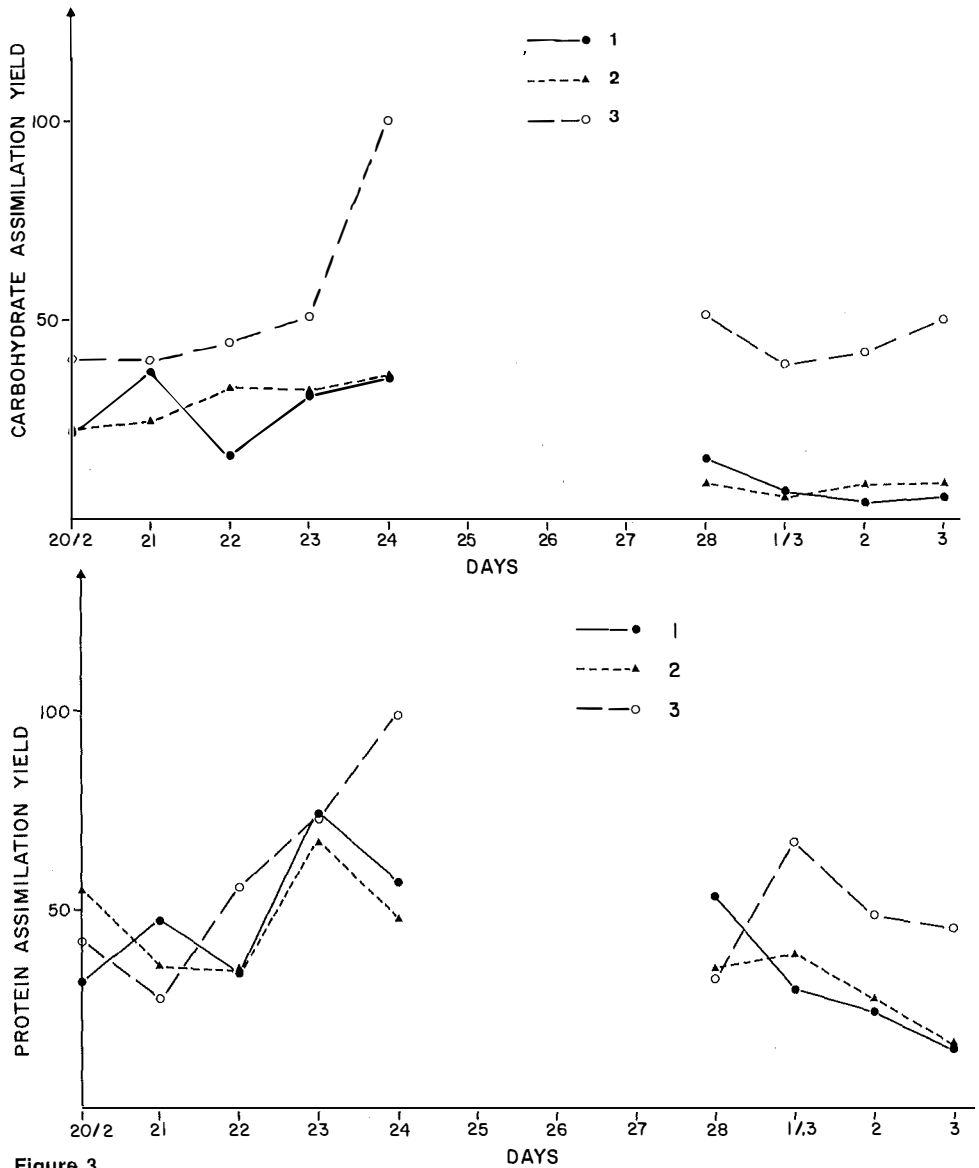
**Figure 2**

Comparison between experiment (1–2) and (3): carbohydrates or proteins

- ingested exp (1–2)/ingested exp (3)
- assimilated exp (1–2)/assimilated exp (3)

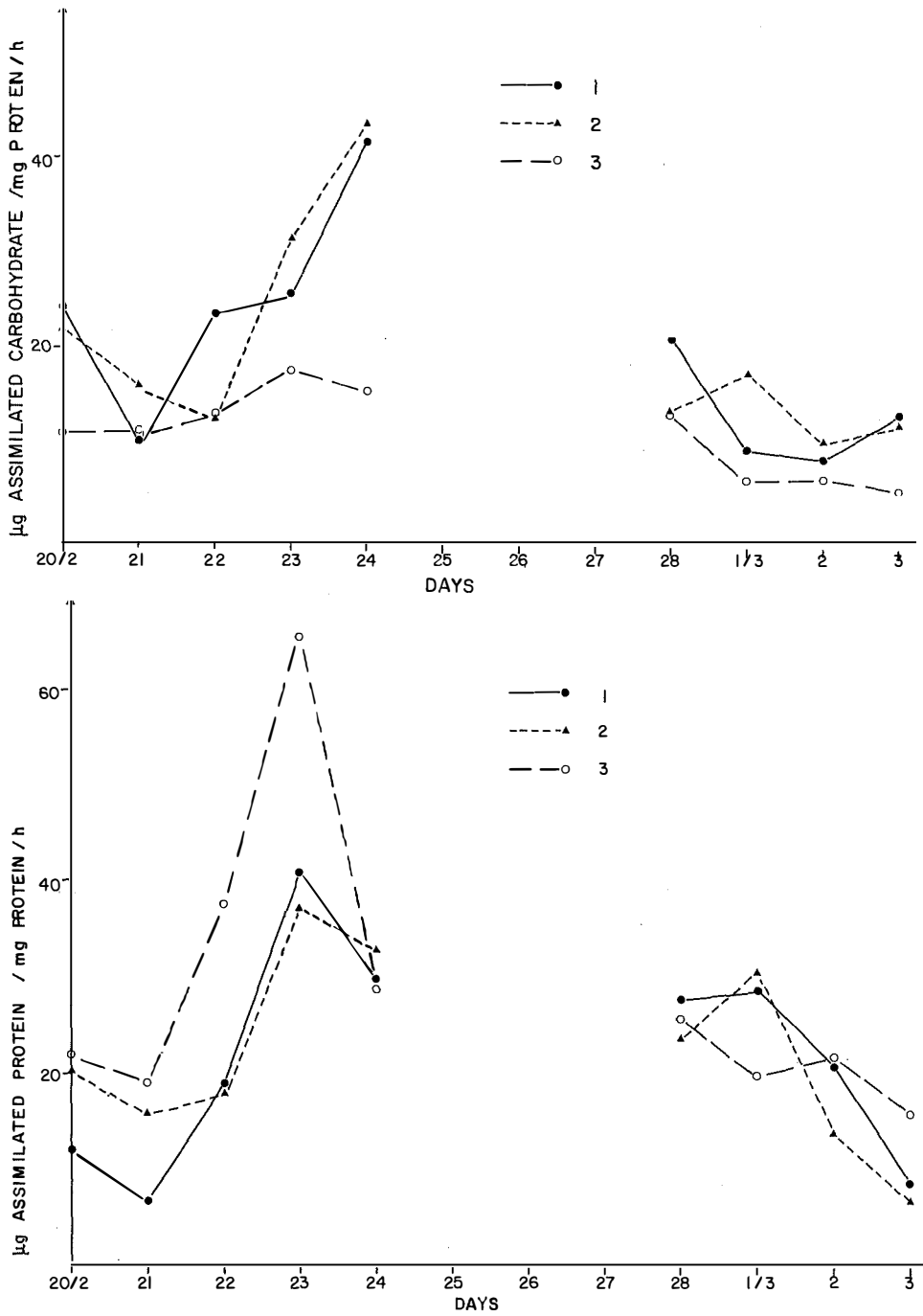
assimilated proteins between the two experiments is no greater when trypsin activities are taken into account.

The yield: The assimilation yield for carbohydrates and proteins has been calculated for the three experiments to provide evidence of the mechanism of a balance in assimilation. The yield for carbohydrate and protein assimilation are reported in Fig. 3. Experiments 1 and 2 give very similar results for the two components. The assimilation yield for carbohydrates from exp 1 and 2 is between 5 and 35 %. The yield is higher for



**Figure 3**

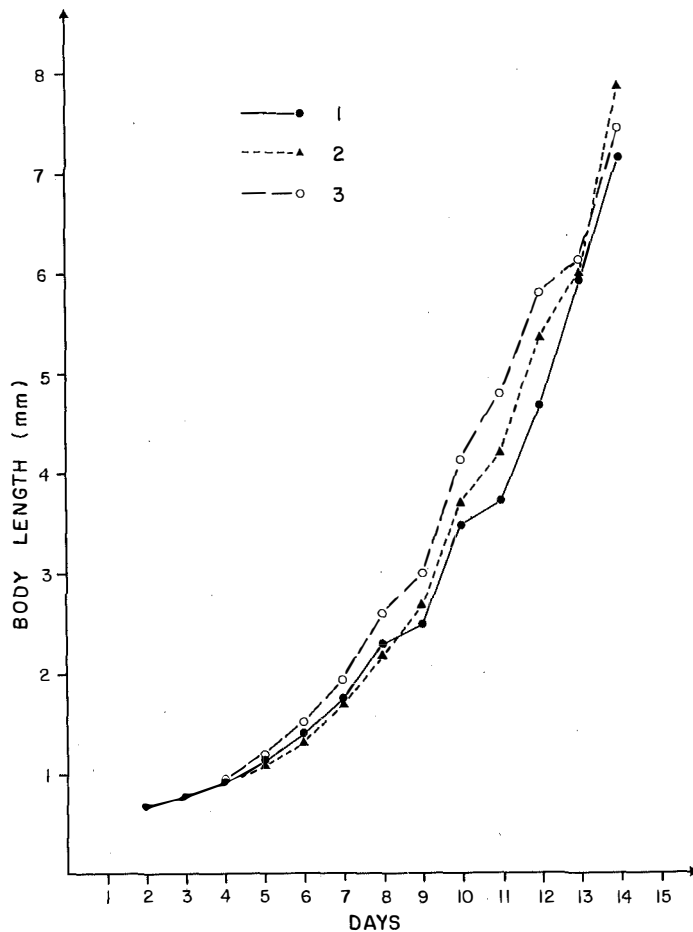
Assimilation/ingestion yield as a function of the growth (in days after hatching) a. Carbohydrates  
b. Proteins



**Figure 4**  
 Assimilation/*Artemia* protein mg/hour as a function of the growth  
 (in days after hatching) a. Carbohydrates  
 b. Proteins

experiment 3 (40 to 100 %). The yield for proteins is identical at the beginning of the three experiments (30 to 70 %). It is higher at the end of the growth for exp 3 (50–70 % exp 3, to 40 % exp 1–2). During the growth, the yield for carbohydrates and proteins varies. It increases after 3 days (length  $\approx 1.2$  mm) and decreases after the 9th day (length  $\approx 4.0$  mm).

Assimilation result: Carbohydrates and proteins assimilated are shown in Fig. 4. Experiments 1 and 2 give similar results both for proteins and carbohydrates. Assimilated carbohydrates in experiment 3 are the same as in experiment 1 and 2 at the beginning of the growth period (day 2 and 3), and they are 50 % less than in the latter two experiments after the 4th day. Assimilated proteins are higher at the beginning of the exp 3 in comparison with exp 1 and 2, but they are identical in the three experiments after the 9th day. The assimilated proteins and carbohydrates also vary with growth with a higher assimilation occurring during the exponential phase.



**Figure 5**

Body length (mm) as a function of days after hatching



Growth result: (Fig. 5) Comparing these three experiments, the growth rates are roughly the same. Elsewhere, a more detailed analysis shows that the growth of exp 3 has been significantly higher at the beginning and lower at the end in comparison with exp 1 and 2 which gave similar results.

### Discussion

We have observed that the grazing rate of *Artemia* on the same phytoplankton species is highly modified when chemical composition of the cells is different (MOAL, in preparation). Mainly, cells that are rich in proteins and poor in carbohydrates were grazed at a lower level than the others. There is therefore a balance in proteins ingested in the two experiments and a very important difference in the quantity of carbohydrates ingested (Fig. 2). As the growth rates are similar, either carbohydrates were not limiting, or another mechanism compensated for the low carbohydrate intake resulting from the different grazing rates. We have attempted to test this second hypothesis by the study of the yield and the amount of the carbohydrates and proteins assimilated with regard to the varied quantities of ingested food observed by MOAL.

The digestive enzymes: The different observed ingestion levels, induced by the different chemical composition of the cells are roughly balanced by the regulation of digestive enzymes. *Artemia* is able to obtain the same final quantities of carbohydrates and proteins assimilated by varying the activity of digestive enzymes when large variations of ingested products occur.

This regulation is particularly strong for carbohydrates as ingestion differences of 15 times between experiment 1–2 and experiment 3 are attenuated to a factor of 2 when assimilation is considered (Fig. 2). The activation of amylase explains the observed increase of assimilation yield in experiment 3 (Fig. 3a), to obtain an equivalent quantity of carbohydrates assimilated in the three experiments (Fig. 4a).

The same process takes place for proteins, but to a lesser extent because ingestion differences between experiments are less important.

Saturation levels: Taking into account the fact that carbohydrate assimilation yield from exp 1 and 2 is always lower than in exp 3, assimilated carbohydrates from these two experiments would be at saturation level for the different stages of the organisms. Therefore these levels could be an indication of the different requirements of *Artemia* during their growth. In exp 3, this carbohydrate saturation level would be obtained only on the second and third day. In the same way, protein assimilation yield decreases only in exp 1 and 2 at the end of the growth. We conclude that apart from this last period, proteins were never at a saturating level. Furthermore, during the first days, assimilated proteins are higher in exp 3. This is proof that in exp 1 and 2 assimilation of proteins is limited. Otherwise, the growth rates are nearly comparable with our preceding experiments (SAMAIN et al. 1980) indicating that the difference between the saturation levels obtained for carbohydrates (exp 1–2) and the levels obtained in exp 3 is not very decisive. On the same basis, even if proteins do not seem to be at saturation level at the beginning of growth, the assimilated amounts produce a growth rate consistent with conditions which are not far from saturation level. If the protein requirements were very much higher than the observed assimilated proteins, we could not explain why the yields from exp 1 and 2 approaching 50 % were not better. The 50 % yield seems to be an usual yield (MARSHALL and ORR 1955, 1956, CONOVER 1968, SUSCHENYA 1970, LASENBY and LANGFORD 1973, LASKER 1973, MOOTZ and EPIFANIO 1974, COSPER and REEVE 1975, PETIPA 1978, PECHENIK and FISHER 1979).

Assimilation and growth rate: We can deduce an explanation of the differences in growth between experiment 1–2 and experiment 3 by comparing the assimilated proteins and carbohydrates. During the first step of the growth (day 1 to 3) assimilated carbohydrates are similar in the three experiments, but assimilated proteins are higher in exp 3. As a result the growth during this experiment was better. During the second step of growth (days 4 to 12) assimilated carbohydrates are lower in exp 3 even with a 50 % yield, but no difference is detected for proteins from day 9 to 12 between the three conditions. As a result, during this period the growth rate of exp 3 decreases in comparison with experiment 1–2.

Assimilation and stages: Another result should be pointed out: Assimilation yield and in consequence the assimilated products vary all along the growth. In the three experiments, the yield increases in a first step and then decreases, corroborating preceding results (SAMAIN et al. 1980).

Different requirements, related to stages, can explain these variations. In this case the first regulatory factor for nutrition processes would be the requirements of organisms. The synthesis of digestive enzymes would be regulated in response to the requirements according to the chemical composition of the food ingested.

#### Requirements:

The results presented here allow a new concept in nutrition studies if requirements are the first internal factor that affects feeding behaviour. One requirement situation will be satisfied depending on environmental conditions. Two hypotheses are possible:

1. The trophic environment is limiting: In this case, organisms will tend to satisfy their requirements by optimizing ingestion and digestion processes. Ingestion will be regulated mainly by the food concentration in accordance with the most numerous size of particles (in the range of size corresponding to the organisms) (POULET 1974), and digestive enzymes will be synthesized to obtain the highest possible yield of assimilation.

2. The trophic environment is saturated: As a balance can be performed by way of the ingestion and digestive enzyme synthesis, the most adequate system will incorporate responses to different possibilities of ingestion, according to the concentration, the taste of particles (MOAL, in preparation), a preferential size, the phytoplankton species, and the corresponding digestive enzyme synthesis in accordance with requirements.

It is probable that the most adequate system will also take the energetic cost in relation to the energy assimilated into account.

All these results show that the production model will change with the requirements of the organisms. As assimilation yields vary with stages or with chemical composition of the food, a production estimate cannot be performed with ingestion rates alone. Digestive enzyme activity must also be taken into account and the saturating or limiting potential of the trophic environmental conditions to satisfy the requirements of organisms must be studied.

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