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Influence of food organisms on the development and culture of pelagic copepods*

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EXTRAIT: **Influence des organismes alimentaires sur le développement et la culture de copépodes pélagiques.** Au cours d'expériences de nutrition réalisées sur *Euterpina acutifrons*, 16 espèces d'algues ont été comparées. Dans les cultures débutées avec des copépodes adultes, seules 4 algues permettent à la population de s'accroître à un taux comparable à ce qui est obtenu lorsque les algues sont en solutions plurispécifiques. Dans les cultures débutées avec des oeufs, certaines algues qui permettaient à une population adulte d'évoluer ne sont pas favorables au développement complet de l'animal à travers ses stades juvéniles. L'influence de la concentration en nourriture dans le milieu de culture a été déterminée pour 5 espèces d'algues. Une relation directe peut être mise en évidence entre la concentration en nourriture dans le milieu de culture et le taux journalier d'ingestion ou le taux de production d'oeufs chez *Euterpina acutifrons* adulte. Le taux journalier de filtration est en relation inverse. Taux d'ingestion journalier et taux de production des oeufs atteignent un plateau qui se situe à un niveau différent et est atteint à une concentration variable pour les 5 algues expérimentées. Ces résultats permettent de mettre en évidence l'importance de la taille des cellules d'algue, de leurs qualités nutritionnelles et de leur concentration; ces différents paramètres influencent la propagation du copépode en culture. De fait, lors d'essais de mise en culture de nouvelles espèces de copépodes, le nombre d'algues et leur concentration furent accrus dans les solutions; ces conditions nous ont permis de maintenir en laboratoire à travers plusieurs générations certains calanoïdes pélagiques tels que *Centropages typicus, Acartia clausi, Ctenocalanus vanus* et *Clausocalanus acuicornis* en plus de deux harpacticoïdes: *Euterpina acutifrons* et *Tigriopus brévicornis.*

INTRODUCTION

In order to investigate the uptake and loss of radio-isotopes in a zooplankton community, it is necessary to conduct experiments carried out under precisely controlled conditions in the laboratory. Planktonic copepods form an essential element in the marine food chain; they are known to be difficult to keep under laboratory conditions and it is only recently that a few species have been bred through several generations.

Of the pelagic calanoids, only *Calanus hyperboreus* (CONOVER 1962) and a few

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individuals of *Calanus nasutus* (MULLIN & BROOKS 1967) have produced one filial generation in the laboratory; *Acartia clausi* have been reared also (CORKETT & URRY 1968); the coastal calanoids *Acartia tonsa* (ZILLIOUX & WILSON 1964), *Pseudodiaptomus coronatus* (JACOBS 1961) and *Eurytemora affinis* (HEINLE 1969) have been reared for a few generations. Harpacticoid copepods, of course, can readily be cultured: some species of the coastal genus *Tigriopus* have lived for years in artificial conditions (FRASER 1936, PROVASOLI et al. 1959, GILAT 1967) and the coastal *Tisbe furcata* (BARR 1969) or the pelagic *Euterpina acutifrons* (BERNARD 1961, NEUNES & PONGOLINI 1965) produced numerous generations under laboratory conditions.

Numerous experiments (CORKETT & MCLAREN 1969) showed that certain physical factors, such as temperature, salinity, light, etc., may have an influence on the survival, reproduction and other metabolic activities of the copepod in culture conditions. Bacterial pollutions were also described as a possible cause of failure in the culturing of zooplankton organisms. The basic parameter, however, seems to provide copepods with food suitable in quality and quantity which allow them not merely to survive but to develop and to reproduce.

Selecting a priori this type of food, for organisms which cannot be kept under laboratory conditions, is difficult; only indirect information such as ecological ones or survival experiments will determine the selection of food.

In their attempt to rear copepods under laboratory conditions, most of the authors provide the animals with grazing media made up of one or, at the most, 5 species of algae. PROVASOLI et al. (1959), however, investigating on *Tigriopus brevicornis* the nutritional value of about 20 flagellates through the entire life span from egg to adult of successive generations, concluded that the nutritional value of these algae differed widely: some species do not allow reproduction at all; others do not allow development to adulthood; others support only a fairly limited number of consecutive generations; the presence of bacteria or micronutrients in the various algal solutions, or solutions with mixed algae, supported more generations than did the monaxenic solutions. The authors concluded that this variable ability of the copepod to utilize the various species of algae did not depend on the size of the algae (which varies only between 6 and 15 μ) but on the presence of certain factors such as micronutrients or vitamins. Unfortunately, in the experiments of PROVASOLI et al. (1959) the range of variation in the size of the algae was not very large and did not enable the importance of the size of an algae in the rate of propagation of the copepod to be determined. No evidence was available that these algae were all ingested by *T. brevicornis* in quantities large enough at each development stage. On the other hand, no data was available on the concentration of the various algae in the food solutions, which are known to have a direct influence on the filtration and ingestion rates, the egg production and the generation time of copepods.

In order to obtain more information on how to provide copepods with suitable food organisms, some points must still receive further attention: (a) availability and/or preference of copepods for certain species of phytoplankton, (b) possibility of a given algae species to support the development of the copepod from egg to mature adult, and (c) efficiency of food conversion.

Some experiments conducted with *Euterpina acutifrons* are presented in this pa-

per and partially answer these questions; we will describe a culture solution which gave satisfactory results for the various species of copepods investigated.

MATERIAL AND TECHNIQUES

The harpacticoid copepod *Euterpina acutifrons* was reared in our laboratory by NEUNES (1965); up till now, these cultures are still alive and healthy after six years (about 100 generations). The life span is short (about 23 days) and concentrations in the culture can reach 10,000 animals/l. All cultures of *Euterpina acutifrons* later initiated with NEUNES (1965) techniques were equally successful. A suspension of a mixture of flagellates *Platymonas suecica, Dicrateria* sp. and *Platymonas* sp., a dinoflagellate *Gymnodinium* sp. and the diatom *Phaeodactylum tricornutum* was added as food organisms.

Sea water is filtered through filter paper and then autoclaved at 120° C and 0.5 atm; an antibiotic (0,1 mg/l penicillin) and a chelating agent EDTA (37 mg/l) are added. Mass cultures of copepods are kept in 20 l Erlenmeyer filled with 10 l of food solution. Permanent air bubbling is supplied in the culture vessels by means of an electric pump. The temperature in the thermostatic room is constant at 18° C (± 1°), under a weak illumination of 12 h/day. The culture medium is changed at regular intervals ranging from 1 week to 1 month, using a siphon fitted with 21 μ pore size nylon gauze. Sometimes, when the dead algal deposit on the bottom is excessive, some of the copepod population is washed and then transferred to a new beaker with a PASTEUR micropipette. In order to start new cultures, copepods are caught in our sampling zone (Ligurian Sea off La Spezia, BERNHARD et al. 1963) with a 180 μ pore size conic net. They are immediately sorted aboard, then introduced into the medium culture and maintained as far as possible at sea temperature by immersing the Erlenmeyer in a large vessel containing fresh sea water.

The various algae species were isolated and cultivated in our botanical department (BERNHARD et al. 1963).

Techniques for the study of ingestion of food, filtration rate and egg production by *Euterpina acutifrons* were described in detail in a previous work (NASSOGNE 1969). Experiments on food ingestion were conducted in Erlenmeyer filled with 50–200 ml of solution and 5 to 10 copepods, depending on algal concentration, kept for 24 h in darkness. Decreases in algal concentration due to the filtering activity of the copepods are corrected for algal concentration variation in the same volume without copepods. Algal concentrations were determined by UTERMÖHL's method under an inverted microscope (UTERMÖHL 1936).

The filtration activity was calculated as the ratio between the number of algal cells ingested/copepod/day and the initial cell concentration in the medium; it represents the corresponding swept clear volume. In egg production experiments, young adult females were introduced into the nutrient solution at the moment they produced the first egg sac. Released eggs and new hatched nauplii were daily picked out of the solution with a micropipette while being counted.

RESULTS

Influence of quality of food on growth of *Euterpina acutifrons* population

Adults of *Euterpina acutifrons* were reared on 16 individual algae species, on a mixture of the same 16 species and on a mixture of only five of them (NEUNES solution). Some results are shown in Figure 1.

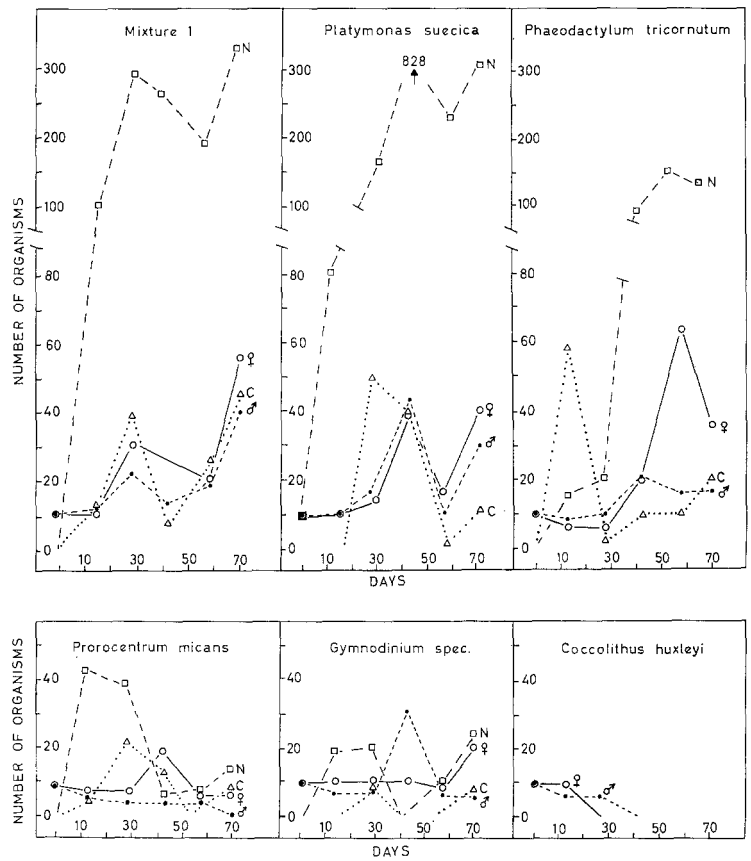

Fig. 1: Growth of a population of *Euterpina acutifrons* fed on various foods. Population started with 10 females (F) and 10 males (M) (N: nauplii. C: copepodits)

The production rates of nauplii, copepodits and adults were widely different, depending on the food organisms. *Phaeodactylum tricornutum, Platymonas suecica, Chaetoceros danicus* and the two mixture solutions only provided a high population growth. Some algae, such as *Coccolithus huxleyi,* did not allow adults to survive. When plotting the production rates of animals against the size of the various algae

in unicellular solutions (Fig. 2), it could be observed that the reproduction rate is high only in the unicellular culture of algae of medium size (between 6 and 16 μ).

Algae smaller than 6 to 7 μ (such as *Coccolithus huxleyi*) or wider than about 16 μ (such as *Gymnodinium* sp. or *Prorocentrum micans*) lowered the rates of population growth.

It seemed that smaller algae could not be filtered by adults or juvenile stages and wider algae could not be ingested by one or all of the development stages.

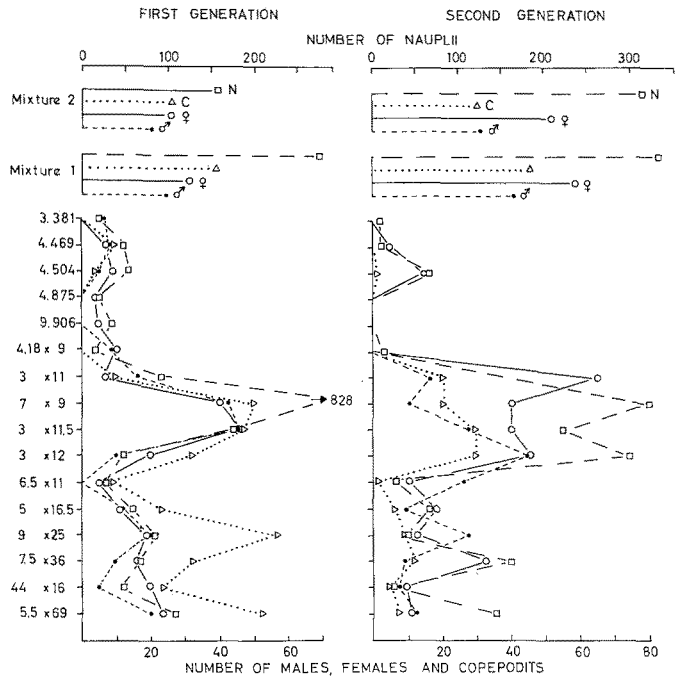

Fig. 2: Relation between size (in μ) of algae (arranged at the ordinate) and number of *Euterpina acutifrons* produced after the first and the second generation (N: nauplii, C: copepodits)

Experiments on the quantity of food ingested by an adult *Euterpina acutifrons* female fed on various unicellular algae at various concentrations confirmed that algae wider than 6 to 7 μ only could be ingested; smaller ones like *Coccolithus huxleyi* were not significantly removed from the solution.

On the other hand, in a series of experiments started with *Euterpina acutifrons* eggs (Fig. 3) it could be observed that algae of medium size only (such as *Phaeodactylum tricornutum* and *Platymonas suecica*) were able, like the mixture of the 13 algae, to support complete development from egg to adult. In solutions of smaller or wider algae, newly hatched nauplii died before reaching the first 4 nauplius stages.

We can conclude that the size of food is one, but may be not the only, limiting factor in the growth of a copepod population.

Relation between the quantity of *Platymonas suecica* removed from a unicellular solution and the production of eggs by *Euterpina acutifrons*

A series of experiments were performed with adult females of *Euterpina acutifrons* and the flagellate *Platymonas suecica* as food organism, in various concentrations (Fig. 4). In these experiments, the number of algae and the corresponding volume swept clear per animal per day were compared with the number of eggs produced during the adult life span of the female.

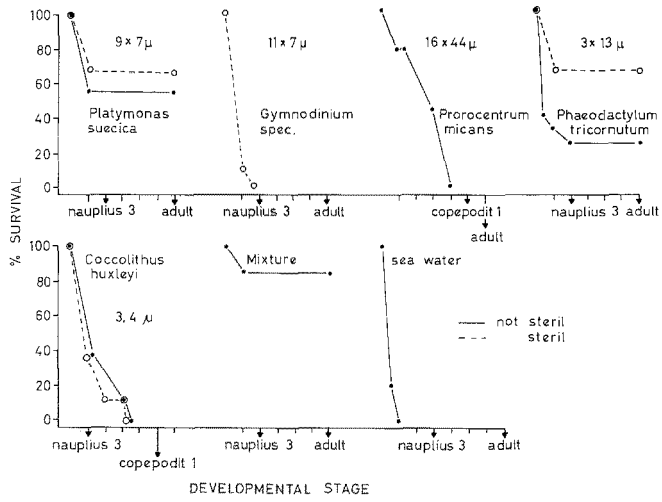

Fig. 3: Development of *Euterpina acutifrons* from egg to adult in various food solutions (N3: nauplius 3, C1: copepodit 1, A: adult)

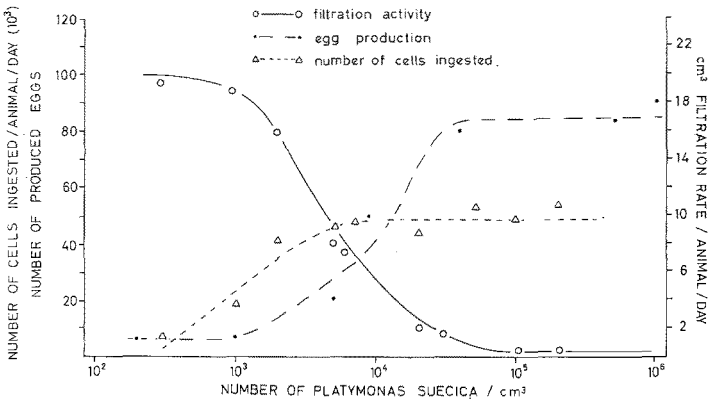

Fig. 4: Influence of *Platymonas suecica* concentration on filtering activity, rate of ingestion and egg production of adult female *Euterpina acutifrons*

As the food concentration increased in the culture medium – so the swept clear volume decreased, while the number of cells ingested increased until it reached a plateau at 5.10^3 cells/ml. Concurrently, the egg production increased and also reached a plateau when the concentration of *Platymonas suecica* came to 10^5 cells/ml. This shows that concentration of algae in the grazing medium influences the egg production and hence the growth of a copepod population; at low concentrations adults could survive but produced very few eggs; high egg production occurred only after the number of cells ingested daily had reached its plateau.

Influence of different algal species at various concentrations on filtration and ingestion activities of adult female *Euterpina acutifrons*

The ingestion of food, filtration activity, and egg production by *Euterpina acutifrons* on various unicellular algal solutions at various concentrations were studied using the techniques described above. The results obtained for the 5 species tested showed again that the increasing of algal concentration in the culture (Fig. 5) caused the quantity of food ingested per copepod per day (expressed here in μg wet weight) to increase until reaching a plateau. However, for equal concentration of algal cells or for equal quantities of algal biomass offered for different species, different quantities of algal biomass were ingested. Furthermore, the concentration at which the plateau is reached and the quantity of food ingested at the plateau were also quite different for each of the 5 algae.

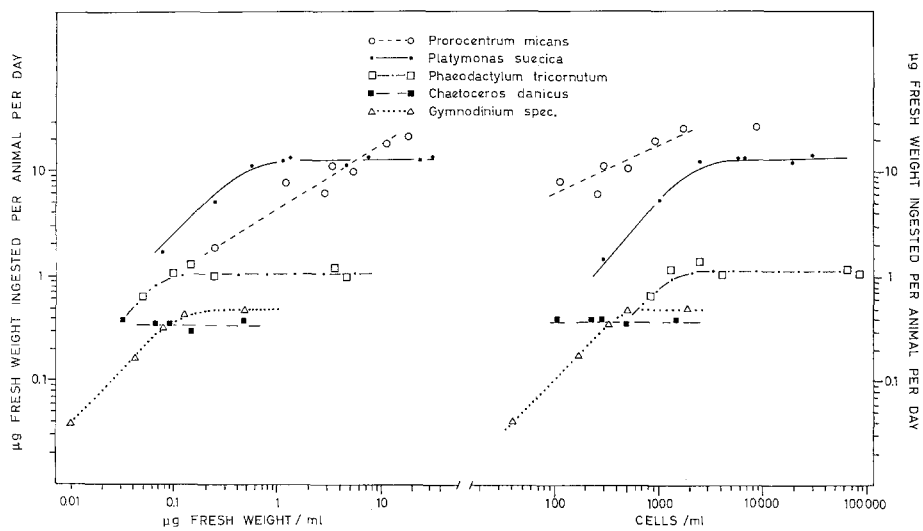

Fig. 5: Influence of food concentration on rate of ingestion of *Euterpina acutifrons* (adult females), fed on different algal species

Similar results were obtained for the filtration rate (Fig. 6) for all 5 algae. The filtration activity decreases with increasing algal concentration, but at the same concentration (whether expressed in biomass or in number of cells/ml) the volume of water filtered per copepod per day was also quite different.

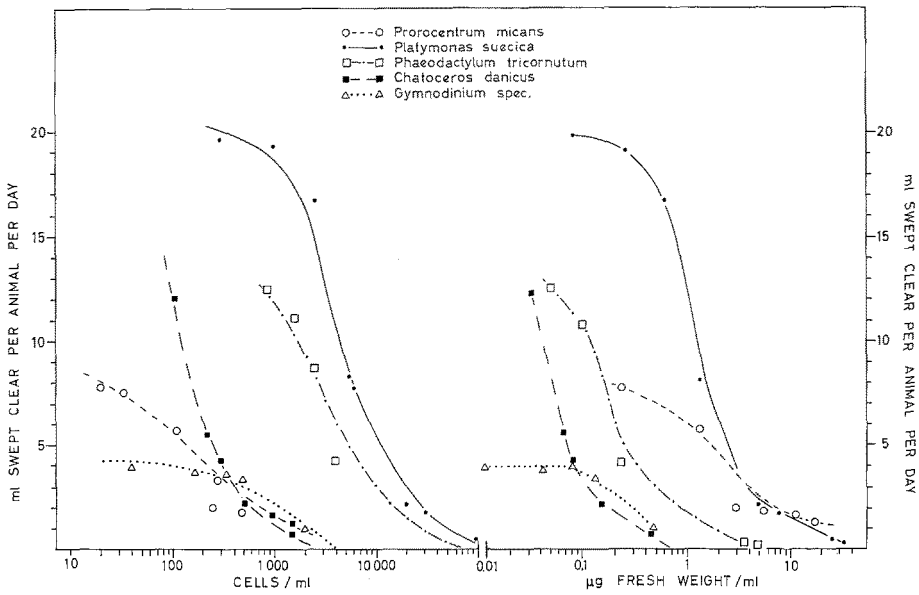

Fig. 6: Influence of food concentration on the filtration rate of *Euterpina acutifrons* (adult female) fed on different algal species

These results showed that apart from food concentration, the filtering activity and the rate of ingestion by the copepod depend also on the quality of the algae. That means that availability of food and the preference of the copepod are important.

Influence of type of food on egg production

Young adult females of *Euterpina acutifrons* were fed in solutions of various algae to measure egg production during their adult life span, i. e. from maturity to death. The concentration of each algae in the unicellular culture solution was chosen to ensure a filtration rate of less than 1 ml/day/animal; under these conditions the daily ingestion was at its maximum and only the nature of the algae could influence the egg production. For comparison, females were also fed with a mixture containing all 5 species. The results showed (Table 1) that the egg production and the adult survival time were different for each species tested.

Prorocentrum micans, although ingested at a rate of 20 µg fresh weight (F. W.)/ copepod/day, showed reduced egg production and adult life span.

Table 1

Egg production and adult life span of *Euterpina acutifrons* fed on different algal species under conditions of excess food

Algae	Culture solution cells/ml	Culture solution μg fresh weight/ml	Total eggs per adult	Days of adult life	Number of experiments
Prorocentrum micans	2.10^3	23.5	14.6 ± 11.3	14.3 ± 2.5	3
Platymonas suecica	1.10^5	26	83.8 ± 6.9	26.6 ± 2.4	7
Gymnodinium sp.	1.10^5	25	135 ± 25.5	36.6 ± 2.5	3
Phaeodactylum tricornutum	4.10^5	24	151 ± 42.2	32.6 ± 7.5	3
Chaetoceros danicus	8.10^4	24	151.3 ± 42.3	27.6 ± 7.7	3
Mixture	3.10^4	24	280.6 ± 17.2	38.3 ± 1.1	3

Phaeodactylum tricornutum, *Gymnodinium* sp. and *Chaetoceros danicus*, which were ingested at a rate from 0.4 to 1 μg F. W., supported more eggs and showed longer survival than *Platymonas suecica*, which was ingested at a higher rate (10 μg FW/copepod/day) than *P. micans*. The best results were obtained when *Euterpina acutifrons* was fed in the mixture of all the species. The egg production was 20 times higher

Table 2

Algal solution provided to copepod cultures on May 11, 1969

Algae	Size (μ)	Volume (μ^3)	Concentration cells/ml	Concentration $10^{-2}\mu$g/ml
Diatomeae:				
Chaetoceros affinis	24.9 × 8.7	1 485 ± 25	1 125	167
Chaetoceros danicus	16.5 × 4.8	299 ± 15	375	11.2
Gyrosigma spectabilis	35.8 × 7.4	1 460 ± 47	125	18.2
Leptocylindr. danicus	69.5 × 5.4	1 615 ± 36	5 000	807.6
Phaeodactylum tricornutum	13.2 × 2.9	60 ± 3	7 125	42.5
Skelectonema costatum	21.1 × 2.5	61 ± 3	2 375	14.4
Flagellatae:				
Chroomonas fragilis	9.7 × 3.9	78 ± 4	3 375	26.3
Dicrateria sp. (β3)	4.5	48 ± 3	3 875	18.6
Platymonas suecica	7.2 × 9.4	259 ± 21	1 250	32.4
Platymonas sp. (β43)	4.5	49 ± 3	3 375	16.6
Peridineae:				
Gymnodinium sp.	11.1 × 6.5	247 ± 16	125	3.1
Prorocentrum micans	44.2 × 24.3	11 671 ± 244	125	145.9
Coccolithophorideae:				
Coccolithus huxleyi	3.4	20 ± 2	1 375	2.9
Σ			29 625	1 307

than in *P. micans* and about twice the number in *Gymnodinium* sp., *C. danicus* and *P. tricornutum.*

The survival time was also higher in the mixture. These preliminary results showed that the algal species which allowed a low egg production and a short adult life span were most highly ingested in biomass.

Culture of other species of copepods

Using 13 species of algae of different size and quality (Table 2) at a concentration for which *Euterpina acutifrons* ingested a daily quantity of food corresponding to its plateau, we were able to rear in culture 4 species of pelagic copepods through several generations. Some preliminary results are shown in Table 3.

Table 3

Preliminary results (obtained in 1969) on culturing species of copepods

Species:	*Acartia clausi*	*Tigriopus brevicornis*	*Centropagus typicus*	*Clausocalanus acuicornis*	*Ctenocalanus vanus*
Start of culture	February 7	February 7	May 12	May 12	June 12
Number of copepods	150	80	300	300	200
1st nauplius production	April 22	February 20	June 2	May 20	June 20
Adults alive	~ 50	no observation	~ 50	~ 200	~ 50
Number of generations	4	6 (?)	2	1	2
Generation time (days)	~ 30	~ 30	~ 60	?	~ 35
Adults alive at 25. VIII	~ 2000	~ 200	~ 50	0	~ 10

DISCUSSION

It is the aim of this paper to point out the influence of food organisms on the development and culture of copepods under laboratory conditions. Our results show the importance of several factors.

Size of the algae

The results showed that the first parameter limiting the number of algal species which support all development stages of *Euterpina acutifrons* is the size. It was de-

monstrated for *Calanus* (MARSHALL & ORR 1956, GAULD 1964) that algae narrower than 10 μ were not retained by the setae; a minimum retention size of about 6 to 7 μ was determined for *E. acutifrons*. Algae wider than about 16 μ supported adult survival but did not allow development from egg to adult. In an attempt to rear new copepod species in culture, experiments on adult survival conducted with a view to determining the suitability of food are insufficient. It seems advisable to provide copepods with various algae of different sizes in mixture and to consider the suitability at various development stages

Food concentration

The influence of food concentration on ingestion activity, filtration rate, survival time and egg production was reported by numerous authors (MULLIN 1966, ADAMS & STEELE 1966, CONOVER 1966a, b, 1968, CORKETT & MCLAREN 1969). It is not our intention to discuss here the problem of *Euterpina acutifrons* nutrition; we merely wish to point out that, at low concentrations of food, a copepod can survive or also reproduce, but high egg production occurs solely in excess food concentration after the maximum ingestion and low filtration activity are reached.

Type of food

Filtration activity, rate of ingestion, adult survival time and egg production are influenced by the type of the algae in solution.

Some authors (MULLIN 1966, MULLIN & BROOKS 1967), measured for *Calanus hyperboreus* higher filtering rates on wider algae. In our experiments, filtering activity did not seem to be correlated to the cell size or the group to which the algae belonged.

It is difficult to explain the wide differences between the 5 algae tested when comparing the maximum biomass ingested daily. Some hypotheses can be made which are to be verified in further experiments.

CONOVER (1966) discussed two different possibilities for copepods catching their food: filtration or handling cells. The process of ingestion can be different for the same animal depending on the algal food offered. Some cells, removed from the solution, can be partially ingested; this would explain why for some algae, the volume filtered and the biomass ingested daily could be overestimated. A possible difference in food assimilation must also be investigated. It appears, however, that for the 5 algae tested, a maximum of ingestion was reached; in these conditions of excess food, egg production and survival time differed depending on the type of food offered. An inverse correlation seemed to exist between the maximum quantity of food ingested and the number of eggs produced. These results could be interpreted as being due to a difference in the nutritional composition of the algae as suggested by PROVASOLI et al. (1959). This hypothesis could explain the fact that with *Euterpina acutifrons* better results were always obtained in algal mixtures which reduces the possibility of a lack of micronutrients.

These results on *Euterpina acutifrons* showed the considerable selectivity of the copepod among the various species of algae regarding size, type and concentration. In any attempt to rear new copepod species under laboratory conditions, it seemed advisable to provide copepods with a wide range of algal species in nutrient solution. The first positive results obtained with some pelagic species indicated that the animals were able to find the right type of food in the plurialgal solution offered. This is now subject to determination in further experiments conducted on *Euterpina acutifrons*.

SUMMARY

1. Growth rates of populations started with adult *Euterpina acutifrons* fed on 16 different unicellular algae, and 2 mixtures of these algae, were compared. Only algae of medium size can support growth rates as high as those obtained in the mixture. Smaller algae (6–7 μ) or wider ones ($>$ 16 μ) give lower rates or do not support adult survival.
2. Of 5 algae tested, only those of medium size support all developmental stages from egg to adult of *Euterpina acutifrons*. These results show that the size of food is one of the factors limiting the production of copepods under laboratory conditions.
3. By feeding adult females of *Euterpina acutifrons* on *Platymonas suecica* at various concentrations, it was demonstrated that, when the food concentration increases, the rate of ingestion and the egg production of the copepod increases thereby reaching a plateau, while the rate of filtration decreases.
4. The same relation is found for the 5 algae tested in regard to ingestion and filtration activities. However, for the 5 algal species, the concentration at which the plateau is reached, and the value of ingestion at this plateau, are quite different.
5. When feeding female *Euterpina acutifrons* in excess unicellular solutions of the 5 algae or a mixture of all the algae used, egg production and adult life span differs according to the type of the food algae. Algal species which result in low egg production and short adult life span are most intensively ingested in terms of biomass.
6. By increasing the number of algal species and their concentration in the culture solution, we were able to cultivate some additional pelagic copepods through several generations.

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