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New aspects of the use of inert diets for high density culturing of brine shrimp

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Abstract

Brine shrimp being non-selective filter-feeders, cheap agricultural waste products should be evaluated as potential food sources for their controlled mass culturing.

Preliminary results of screening tests performed with wheat bran, soybean meal, rice bran and whey powder in culturing systems of various sizes are reported. Wheat bran appears to be unsuitable as monodiet for brine shrimp. The other products tested support good growth although specific precautions have to be taken with some foods to avoid problems of water quality during culturing.

Processing techniques for the manual or mechanical preparation of inert feeds for *Artemia* are indicated.

The possibility of influencing the fatty acid pattern of cultured brine shrimp by varying their diet is briefly outlined.

Introduction

The 11 pairs of thoracopods of an adult brine shrimp fulfill several functions : the oar-like endopodites serve for locomotion, the epipodites have a gill-function and the setae on the exo- and endopodites filter small particles from the surrounding medium.

The thoracopods move back and forward without interruption to assure an efficient respiration ; as a result the animals are swimming continuously. The thoracopodal movements induce two water currents along the ventral body surface (Cannon and Leak, 1933 ; Fig. 1) : by moving the limbs towards the head, the interlimb space increases and water is sucked into the midventral groove ; the inner lobes, feathered with dense filtering setae along the margin, collect particles from this incoming water stream. On the backstroke, water is forced out of the interlimb space. The collected particular material is transferred to the midventral food groove and pushed forward by slight spurts of water each time a limb shifts from backstroke to forestroke. In the head region, food particles are packed into a food bolus by a secretion product of the labrum. This bolus is finally pushed into the mouth opening by the action of the first maxillae (Ivleva, 1969).

[4] "Bevoegdverklaard Navorser" at the Belgian National Foundation for Scientific Research (NFWO).

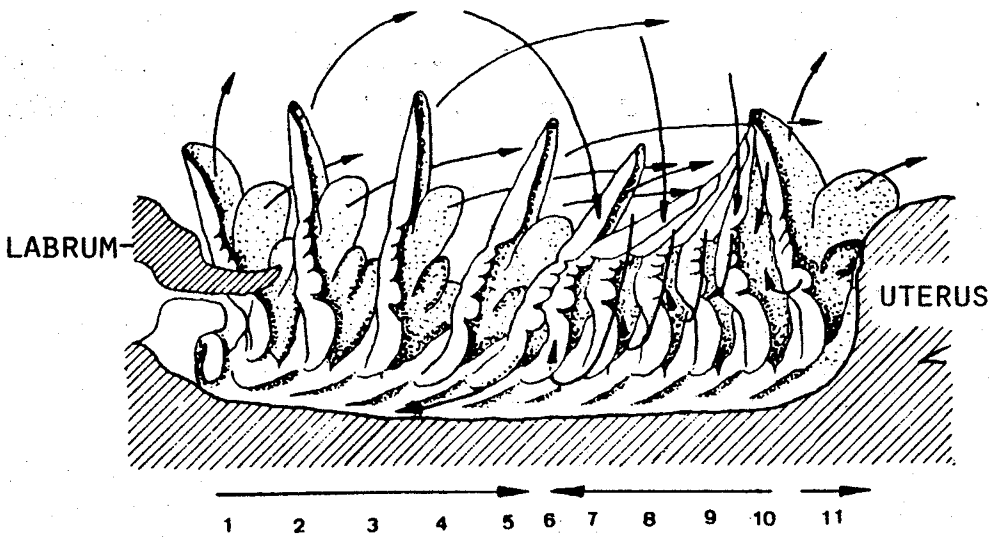

FIG. 1. Schematic diagram of the left row of thoracopods with indication of their separate movements in an adult anostracan shrimp (after Völlmer, 1952). In this phase of movement, thoracopods 1 until 5 and 11 provoke the animal's propulsion whereas the others create an underpressure in the interlimb spaces ; as a result water in sucked in and particles retained on the setae of the exo- and endopodites.

As compared to other Crustacea, *Artemia* has a very primitive feeding mechanism, it is indeed a continuous, non-selective, obligate phagotrophic filter-feeder (Provasoli and Shiraishi, 1959 ; Barker-Jörgensen, 1966). Suspended particles of suitable size, no matter what their nature is (Reeve, 1963), are continuously removed from the culture medium by the beating of the thoracopods. Except for the first larval stages, soluble products cannot be efficiently ingested and as such do not support growth in *Artemia*.

In the early larval stages, the filter-feeding activity of brine shrimp is not very efficient (Tobias *et al.*, 1979) : until instar VI only one pair of appendages, namely the second antennae have a locomotory and filter-feeding function. As molting proceeds, the thoracopods differentiate and become functional one after another, until in stage XII-XIII the maximal filtration capacity is attained when the 11 pairs of thoracopods are fully developed.

From the literature it appears that various food sources both live and inert, have been successfully used to culture *Artemia* from nauplius to adult (Table I). As far as growth rate and production are concerned, best results have always been achieved with a diet of live algae. Most of these tests have, however, been run at a very small scale *e.g.* in test tubes or petri dishes with but a small number of larvae. For high-density mass culturing of *Artemia*, live algae can hardly be taken into consideration since large scale production of the latter is not yet economically feasible. Similarly most inert food sources have either only been used for small-scale testing or cannot be considered for large-scale application because of their limited local availability and/or high price. In this regard the two algal powders, *Scenedesmus* and *Spirulina*, which we thought earlier to be the solution for large scale *Artemia* production

(Sorgeloos 1973, 1974) are unfortunately too expensive «... la recherche d'une nourriture adaptée à bas prix en est d'autant plus nécessaire» (Person-Le Ruyet, 1976).

TABLE I

List of some live and inert food sources known to support good growth in brine shrimp (information from Provasoli *et al.*, 1959 ; Katsutani, 1965 ; Shimaya *et al.*, 1967 ; Takano, 1967 ; Walne, 1967 ; Wolfe, 1971 ; Sorgeloos, 1973, 1974 ; Villacarlos, 1976 ; Jahnig, 1977)

Live algae :	
Diatomeae :	*Chaetoceros, Cyclotella, Phaeodactylum, Nitzschia*
Chlorophyceae :	*Dunaliella, Chlamydomonas, Chlorella, Platymonas, Stichococcus, Stephanoptera, Brachiomonas*
Chrysophyceae :	*Isochrysis, Monochrysis, Stichochrysis, Syracosphaera*
Dried algae :	*Chlorella, Scenedesmus, Spirulina*
Yeasts :	bakers' and brewers yeast
Various inert products :	wheat flower, fish meal, egg-yolk, homogenized liver, rice powder

In the framework of a consultancy by one of us (Sorgeloos) in 1977 for the Jepara FAO-project in Indonesia and the SEAFDEC Aquaculture Department in the Philippines, agricultural waste products were screened for their potential use as food for large scale production of brine shrimp. Successfull results were reported with coconut meal, sugarcane molasses and soybean cake (Persoone, 1978 ; Talloen, 1978).

In order to optimise the ingestibility of agricultural products by *Artemia*, we have determined the maximum particle size that can be taken up by nauplii and adults. The paper reports culturing results obtained in feeding brine shrimps with rice bran, soybean, wheat bran and whey powder. Data are given on the fatty acid composition of *Artemia* fed various diets.

Relationship of particle size to ingestion in nauplii and adult *Artemia*

Although we know from the literature that brine shrimp ingest particles from a few micrometers in size [bacteria (Seki 1964)] up to close to 25 μm (Takano, 1967) the maximal size of particles which can be ingested by *Artemia* nauplii, respectively adults has never been defined accurately.

In order to answer this important question, the following experiment was carried out : instar III-IV nauplii (Hentschel, 1968) and adult brine shrimp from San Francisco Bay (California, USA) were offered a mixture of glass microspheres[5] ranging in diameter from a few micrometers up to 120 μm. Plastic test tubes of 5 ml were filled with natural seawater, inoculated with nauplii or adults and given a small amount of glass microspheres. The stoppered tubes were mounted on a rotating device (5 rpm) in order to keep all particles in

[5] Made available by N.V. Glaverbel, Belgium.

continuous suspension. Once the digestive tract was filled with microspheres and before defecation had started (determined to be 3 min for adults and 10 min for nauplii), the animals were fixed with a few drops of lugol's solution. The size determinations of the ingested particles were performed with a microscope equiped with a calibrated eye-piece. For the nauplii the ingested microspheres could be observed easily through the transparent cuticula but for the adults the digestive tract had to be removed by dissection.

In total about 3 500 microspheres have been measured, 2 000 of which were sampled at random from the test tubes, 1 000 ingested by 30 nauplii and 800 ingested by 5 adults.

The size frequency-distribution of the microspheres in suspension in the medium and those ingested by the instar III-IV nauplii, respectively the adults, are represented graphically in Fig. 2 and 3.

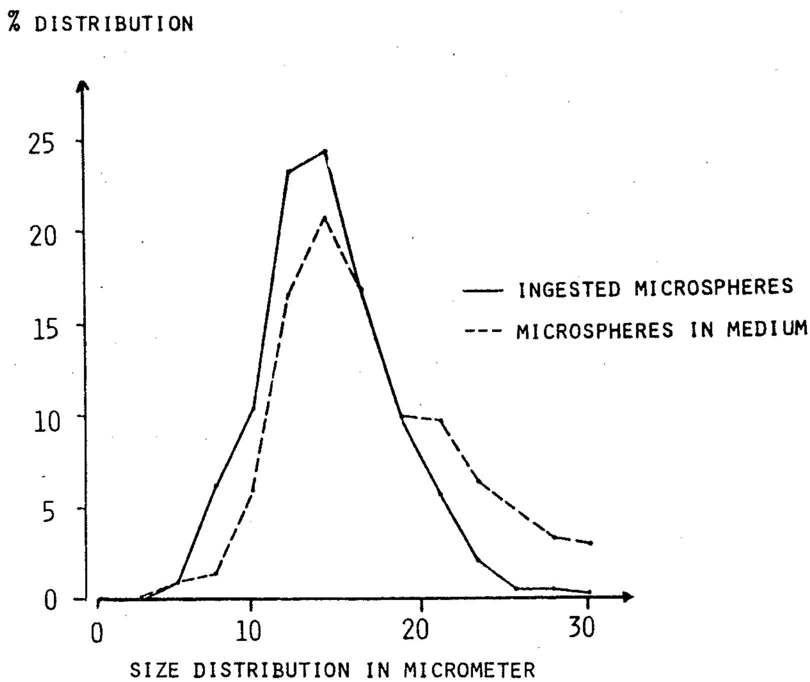

FIG. 2. Size frequency distribution of microspheres present in the medium (broken line) and ingested by instar III-IV nauplii (straight line).

From these results it clearly appears that the maximal size range of particles that can be ingested is 25 to 30 μm for nauplii and 40 to 50 μm for adult brine shrimp. It is interesting to note that there is much overlapping in the size distribution curves which is a further proof of the non-selective nature of the feeding process in *Artemia*.

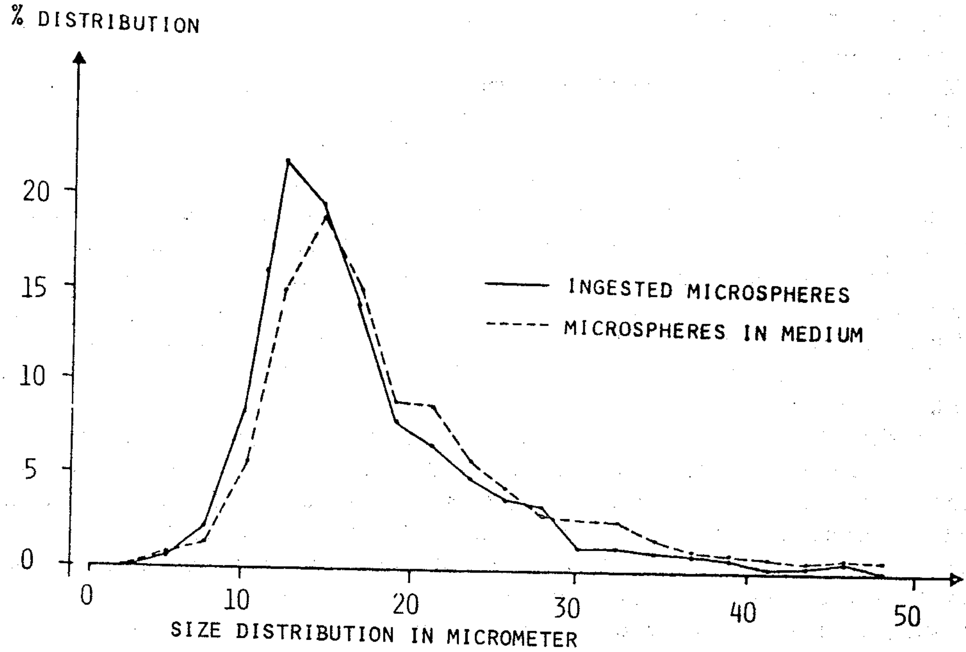

FIG. 3. Size frequency distribution of microspheres present in the medium (broken line) and ingested by adult brine shrimp (straight line).

Evaluation of rice bran, wheat bran, soybean- and whey powder as food for brine shrimp

Initial screening tests were performed in cylindrical-conical glass tubes (10 cm in diameter) : 200 nauplii were incubated in 600 ml natural seawater in each 1 l tube at a constant temperature of 28 °C. In order to assure good oxygenation of the culture medium and to keep the food particles in suspension an intermittant air bubbling from the bottom of the tubes was applied every 3 min for 5 sec. Food suspension was made up as follows : 50 g product was suspended in 1 000 ml seawater, homogenized and passed over a 50 μm screen. Since it could be expected that the quantity of food for optimal growth and survival varies from one type of food to another, the relative value of each product was determined by feeding different amounts of stock suspension in five series of replicate tests. Food was provided twice a day and the culture media renewed every second day. After 10 days culturing the number of survivors was determined. The larval length was measured using a dissection microscope and camera lucida projection. For all cases in which the survival rate exceeded 50%, raceway-culturing tests were set up following the technique described by Bossuyt and Sorgeloos (1980) to further evaluate the suitability of the respective food sources for mass culturing of brine shrimp.

Poor results were obtained with wheat bran : maximal survival was less than 10% after 10 days and the larvae reached a length of only 2.08 mm ± 0.39 mm. Those results corroborate the finding of Takano (1967) that wheat bran used as a mono-diet for brine shrimp is nutritionally insufficient.

Much better results were achieved on a soybean-diet : 80% survival and a maximal larval length of 3.24 mm ± 0.29 mm. In raceway cultures growth was also good but within 4 to 5 days batch culturing, high ammonia levels were built up and high mortalities were noted. The explanation is that soybean powder appears to contain high amounts of soluble proteins which cannot be ingested by *Artemia* but provokes water pollution by bacterial development. Soybean powder can, however, be processed into an acceptable food for batch culturing of *Artemia* by treatment of the soybean suspension with a cream separator : the outflow containing the dissolved matter is discarded while the centrifugate is resuspended in brine solution and used as food stock. Using this technology, we have been able to convert the nauplii hatched out of 20 g cysts, into 3.8 kg adult biomass in a 2 m^3 raceway in less than 2 weeks batch culturing at 25 °C.

With rice bran, an average larval length of 4.26 mm was attained at a survival rate of over 80%. As reported elsewhere (Sorgeloos *et al.*, 1980) rice bran is a very cheap product available worldwide which supports good growth in *Artemia* and is very suitable for batch culturing because its contents of water soluble components are minimal. As a result of its fibrous structure, less than 50% of the initial crude product can be processed into *Artemia* food by homogenizing and sieving treatment. In order to reduce the costs of manpower in preparing the food and to develop a storable dry product that can be fed to the *Artemia* without any further manual preparations, we have studied the technical and commercial applicability of dry grinding and processing techniques. The best results were obtained with the Ultrafine® mill, the working principle of which is that a current of ambient air entrains crude rice bran powder into a grinding circuit, where three types of physical action (vortexes, changes in pressure, and vibrations) simultanously act on the rice bran particle, provoking a high degree of autogenous grinding of the material. The application of the Ultrafine® mill for rice bran grinding is new. The best results so far were obtained with defatted rice bran : on a weight basis, over 80% of the grounded product has a particle size inferior to 60 μm. The present price of micronised rice bran still amounts to U.S. $ 1.5/kg. Further automatization of the processing should, however, lead to a significant drop in the final price.

The best production results attained in batch culture in air-water-lift raceways were obtained with rice bran : on the average 2 kg *Artemia* biomass are produced per cubic meter of water within a period of 2 weeks, at 28 °C. This culture technique of *Artemia* on rice bran is, by our knowledge, already applied by aquaculture laboratories in Brazil, France, India, Indonesia, Norway, the Philippines, Thailand, the USA, and Venezuela. It is important to add here that although most *Artemia* strains which we tested so far grow well on a rice bran diet, we have been informed that other strains do not thrive on this sole diet : *e.g.* San Pablo Bay (California, USA, Bernardino, personal communication ; Abreu-Grobois, personal communication, and own observations), Lavalduc-France, Eilat-Israel, and Izmir-Turkey (Abreu-Grobois, personal communication). As a result it becomes imperative to study the differences in nutritional requirements between geographical strains of *Artemia*.

Although in earlier feeding tests we had never found a significant difference in food value between crude and defatted rice bran we recently experienced high *Artemia* mortalities after switching to a new commercial lot of crude rice bran. In the meantime we have been informed that depending on the geographical origin, rice bran is sometimes treated with pesticides for transportation and storage. The good results which we always obtain with defatted rice bran can probably be explained by the fact that pesticides are eliminated during defattening of the product.

We have recently discovered a new interesting source of cheap *Artemia* food, namely Lactoserum or whey powder.[6] This product is the particulate matter resulting from drum drying and conditioning of whey suspension in cheese manufacturing. Although the chemical composition of Lactoserum probably varies from one dairy industry to another, whey is usually rich in carbohydrates (60 to 65 % of the dry weight, mainly lactose) but poor in proteins and fatty acids. Its particle size can easily be reduced below the 50 μm upper size limit by hammer mill treatment. In the 600 ml culture tests an average length of 4.6 mm was attained after 10 days culturing. Survival was, however, only 50 to 60 %. Repeated tests in 300 l and 2 m^3 raceways have shown that although high production figures can be attained (up to 2.5 kg/m^3 after 3 weeks), the reproducibility is not guaranteed : mass mortalities indeed often occur, especially during the early larval stages. At larval densities below 3 000 animals/l, bacteria and ciliates often develop in high numbers and adversely affect the water quality. Although this aspect needs further study it has already become clear that with whey-powder food dosage is critical and that better chances for success can be guaranteed by starting the cultures at very high nauplii densities.

Fatty acid pattern in cultured brine shrimp

From the literature it is known that fatty acid patterns in marine organisms are strongly influenced by abiotic and biotic parameters, such as temperature, metabolic needs of the animals and not the least dietary composition (review by Conover, 1978). Recent studies by Watanabe *et al.*, (1978, 1979) reveal the dietary need for fish larvae of the following poly unsaturated fatty acids (PUFA), also called essential fatty acids : 18:2ω6, 18:3ω3, 20:5ω3 and 22:6ω3. In view of the importance of these biochemical components in evaluating the nutritional value of *Artemia* cultured on agricultural waste-products, we have compared the fatty acid pattern of *Spirulina*, rice bran and soybean as well as of adult brine shrimp cultured on these food sources. We have also studied the influence of a diet alteration on the changes of the fatty acid pattern in *Artemia*. The *Artemia* were cultured in 300 l raceways at 25 °C. The adult animals were harvested over a 500 μm screen, washed with tap water, freeze dried, and stored under nitrogen. Fat was extracted with a 2:1 mixture of chloroform and methanol (Medwadowski *et al.*, 1971). After esterifying with 5 % sulfuric acid in methanol, the methyl-esters were stored at −10 °C. The fatty acids were separated and analysed on two 7 m stainless steel columns packed with 15 % diethyl glycocol succinate on chromosorb (acid washed, DMCS, 80-100 mesh) using a Varian Aerograph 2400 gas cromatograph equipped with double flame ionisation detector and temperature programmation from 90 °C to 180 °C at 6 °C/min.

The procentual composition of long chain fatty acids in *Spirulina*, rice bran and soybean meal is summarised in Table II. From these figures it is clear that all food sources contain high amounts of 18:1ω9, 18:2ω6 and, with exception of *Spirulina*, 18:3ω3. The fatty acid data for

[6] Made available by N.V. De Gier, Gierle-Belgium.

the nauplii and cultured brine shrimp are given in Table III. In accordance with Watanabe *et al.* (1978) we observed that the PUFA-concentration increases as the animals grow from nauplii to adults. The very significant drop in 18:3ω3 from 27% in the nauplii to a few percent in the adults is, however, surprising. In rice bran fed *Artemia*, the 18:1ω9 and 18:2ω6 concentration have increased. The low content of 18:3ω3 might be caused by its very low concentration in this specific diet. Although high concentrations of 18:2ω6 are present in soybean meal, this PUFA is metabolised into other fatty acids when taken up by *Artemia*. Comparison of the 18:3ω3 levels in the *Artemia* and their respective diets, reveals that its incorporation in brine shrimp is directly related to its availability in the diet.

TABLE II

Procentual composition of unsaturated fatty acids in *Spirulina*, rice bran and soybean meal (expressed as % of total lipids)

Fatty acid	*Spirulina*	Rice bran	Soybean meal
16:1ω7	12.2	0.1	Trace
18:1ω9	3.0	44.9	15.1
18:2ω6	18.8	36.1	56.6
18:3ω3	trace	1.2	6.4

TABLE III

Procentual composition of unsaturated fatty acids in Great Salt Lake (Utah, USA) *Artemia* nauplii and in adults cultured on rice bran or soybean meal (expressed as % of total lipids)

Fatty acid	GSL-Utah nauplii	Rice bran fed *Artemia*	Soybean fed *Artemia*
16:1ω7	4.6	5.3	10.7
18:1ω9	30.1	47.0	33.5
18:2ω6	5.5	25.1	17.8
18:3ω3	27.4	2.2	4.5
20:5ω3	2.8	Trace	Trace

In the last series of experiments we studied the effect of a diet alteration on the PUFA-changes in *Artemia*. After 14 days culturing on rice bran, the medium was renewed and the animals were fed a *Spirulina*-suspension. Samples of brine shrimp were taken before this change of diet and after 1, 2, and 3 days *Spirulina*-feeding respectively and analysed for their PUFA-contents. The changes in procentual composition are represented graphically in Fig. 4. The drastic change in fatty acid pattern over a period of only a few days is remarkable. The drop in the concentration of 18:1ω9 from 55 to 35% is probably a reflection of its availability in the diet (45% in rice bran, only 3% in *Spirulina*). However, in view of its low content in *Spirulina*, the increase in 18:3ω3 can only be explained by synthesis of this component by the adult brine shrimp.

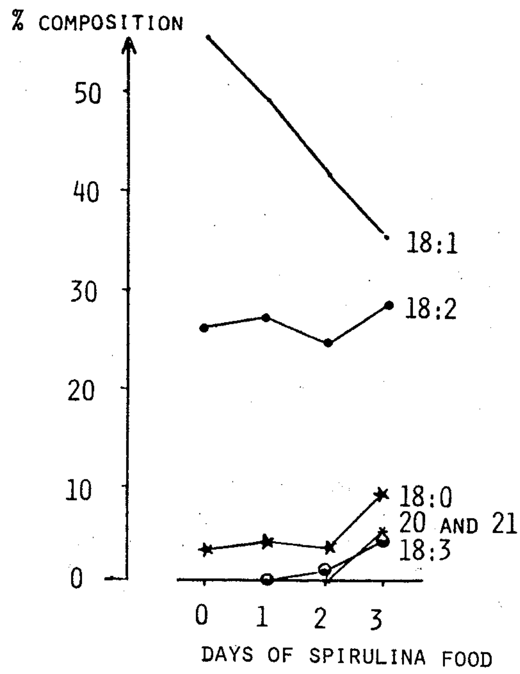

FIG. 4. Changes in fatty acid pattern of *Artemia* switched to a *Spirulina*-diet after 2 weeks rice bran feeding.

Although these data on PUFA composition still need to be completed and interpreted in the light of results obtained in feeding these *Artemia* to fish and crustacean larvae, it is already clear from this experiment that it must be possible to improve the nutritional suitability of *Artemia* through manipulation of the diet. Since brine shrimp can be bulk fed till shortly before their harvest on cheap waste products such as rice bran, a limited feeding period on more expensive formulated diets, should on one hand not have a major impact on the economic feasibility of *Artemia* production, whereas on the other hand it can greatly improve the nutritional value of this invaluable food organism.

Acknowledgement

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