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THE ACCEPTABILITY AND DIGESTIBILITY OF MICROCAPSULES BY LARVAE OF *CRASSOSTREA VIRGINICA*

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ABSTRACT The acceptability and digestibility of microcapsules with gelatin-acacia and nylon-protein walls to larvae of *Crassostrea virginica* were assessed. Larvae were observed to ingest and digest the microcapsules. Gelatin-acacia microcapsules were more digestible than the nylon-protein microcapsules. Results indicated that both types of microcapsules supported some growth of larvae. Larvae fed cod liver oil encapsulated by gelatin-acacia walls grew as rapidly as larvae fed algae. Results also indicated that microcapsule concentration affected growth rate.

INTRODUCTION

A major difficulty in the development of commercial culture systems for molluscan and crustacean larvae is the dependence upon supplies of live organisms for food. This dependence has also obstructed investigations into the nutritional requirements of many bivalve molluscs and crustaceans during their planktonic larval life, although some valuable information about larval nutrition has been gained in the last decade.

Artificial food particles are acceptable to a wide range of filter feeders (Ling 1969, Paffenhofer and Strickland 1970, Jones et al. 1972). However, those particles could be susceptible to disintegration and associated bacterial contamination. One solution to these problems is to use an encapsulated diet. Moreover, if the diet can be defined biochemically, the technique of microencapsulation can be used to investigate the exact nutritional requirements of the animals under culture conditions.

The type of microcapsule that can be used successfully in feeding experiments will be dependent on the mode of feeding of the animals. Bivalve larvae and adults are filter feeders and ingest their food intact. Selection of food particles depends on size, surface properties, and weight of the particle (Ukeles 1971, Owen 1974). Therefore, the test of the acceptability of different types of microcapsules to the animal is important to justify future experiments with encapsulated diet components to evaluate growth and survival. It is also important to demonstrate that microcapsules which are acceptable in terms of ingestion and retention to the bivalves can be digested.

Gelatin-acacia microcapsules are suitable for the presentation of dietary lipids to larvae of *Crassostrea gigas* (Langdon 1980). Previous investigators suggested that lipids play a significant role in the metamorphosis and development of oyster larvae (Helm et al. 1973, Holland and Spencer 1973, Holland 1978, Chu and Dupuy 1980). Consequently, gelatin-acacia microcapsules filled with cod liver oil were used in these feeding experiments. Cod liver oil is rich in highly unsaturated fatty acids (Ackman and Burgher 1964) and has a fatty acid composition quite similar to that found in the protocol algal diet (a combination of *Chlorella* sp., *Pyramimonas virginica,* and *Pseudoisochrysis paradoxa*) used in this laboratory as a standard food source for larvae of *Crassostrea virginica* (Chu and Dupuy 1980).

Jones and his colleagues (Jones et al. 1974, Gabbott et al. 1975, Jones and Gabbot 1976, Jones et al. 1979a, Jones et al. 1979b) successfully encapsulated artificial food particles in nylon-protein microcapsules to study the nutritional requirements of crustacean larvae. It was apparent, therefore, that nylon-protein walled microcapsules could be used to provide protein, lipid, and carbohydrates to oyster larvae. In this paper results of the assessment of the acceptability to and digestibility by larvae of *C. virginica* are reported for microcapsules with gelatin-acacia and nylon-protein walls.

METHODS AND MATERIALS

Microcapsules and Diet

Gelatin-acacia microcapsules were prepared using the methods of Green and Schleicher (1957). Cod liver oil containing vitamins A and D (E. R. Squibb and Sons Inc., Princeton, NJ) was encapsulated for feeding experiments. The mean diameter of the microcapsules was 6.0 ± 1.8 μm ($\bar{x}$ ± SD, n = 25). Stained gelatin-acacia microcapsules were prepared by dissolving Sudan Red in lipid before encapsulation (approximately 1 to 2 mg/ml lipid). Vitamins B_1, B_2, and B_{12} were supplied in the diet by mixing B_1 and B_2 with the lipid and dissolving the B_{12} in the solution of gelatin-acacia prior to microencapsulation. All gelatin-acacia microcapsules, except those fed to the larvae immediately after manufacture, were autoclaved at 121°C (1.053 kg/cm^2 [15 psi] pressure) for 15 minutes and stored in the refrigerator. Autoclaving may have somewhat reduced the vitamin content due to heat lability; vitamin A is considered heat labile at 121°C while vitamins B_1, B_2, B_{12}, and D are not.

Nylon-protein microcapsules were prepared with a modification (Jones and Gabbott 1976) of the polymerization procedure described by Chang et al. (1966). The mean diameter of these microcapsules was 6.1 ± 1.95 μm (n = 25). Whole chicken egg homogenate was mixed with an equal volume of 15% dextrose and 5% cholesterol in distilled water and was incorporated into the nylon-protein microcapsules. Nylon-protein microcapsules were stained by adding Blue dextran to the egg-water mixture (8 mg of Blue dextran for 15 ml of mixture). A summary of the general characteristics of these two types of microcapsules is shown in Table 1.

TABLE 1.

Some properties of gelatin-acacia and nylon-protein microcapsules.

Microcapsules	Gelatin-acacia	Nylon-protein
Size range (diameter)	3 to 8.25 μm	3 to 9 μm
Mean	6.0 μm	6.1 μm
Standard deviation	1.8	1.8
n	25	25
Filling	Cod liver oil and fat-soluble components (e.g., vitamins)	Egg protein or haemoglobin, cholesterol and dextrose
Permeability	Permeable	Semi-permeable
Potential use	To supply lipid, fat-soluble micronutrients	To supply protein, lipid, and carbohydrates

Larval and Algal Culture

Methods used to induce spawning and for embryo culture were those of Dupuy et al. (1977). After spawning, all eggs were pooled and counted before fertilization. About 12.5 x 10^6 fertilized eggs were placed in each 250-l fiberglass larval tank. When the cultures were maintained at temperatures of 27 to 28°C, the larvae reached the straight-hinge stage 18 to 24 hours after fertilization. The methods of Dupuy et al. (1977) were also used in rearing and feeding the oyster larvae.

One algal species, *Pseudoisochrysis paradoxa*, used as part of our protocol diet for bivalve larvae was cultured at 16 to 19°C in 40-l carboys containing filtered and pasteurized estuarine water enriched with N_2M medium (a mixture of Ketchum & Redfield's solution A and B, sodium molybdate solution, Arnon's micronutrient solution), and a horse manure extract mixture (Dupuy et al. 1977). *Pseudoisochrysis* was grown under continuous illumination from one warm white and one Gro-Lux fluorescent lamp. Continuous aeration provided circulation in the cultures.

Feeding Experiment

Two feeding experiments with larvae of *C. virginica* were carried out in the laboratory. Larval density in all feeding experiments was 5 to 6 larvae/ml. Larvae that were fed *P. paradoxa* (the other two species of the protocol diet were unavailable), and starved larvae served as controls for all feeding experiments. Seawater filtered through 10 and then 1 μm Cuno cotton filters was used throughout these experiments.

Feeding and Digestion Activity. Stained gelatin-acacia microcapsules and nylon-protein microcapsules were fed to 2-day-old larvae in 300-ml glass beakers. Microcapsules were fed to larvae each day for 2 days after which the larvae were held for an additional 3 days in clean water. The seawater in the beakers was changed every other day, prior to feeding if the larvae were fed. Beakers containing larvae were covered and held at room temperature (26–27°C). Samples of larvae were observed with a Zeiss standard UPL inverted microscope 24 and 48 hours after the last feeding to observe the contents of the digestive system. Photographs were taken of the same sample after preservation in a 0.5% formalin solution. A Leitz Ortholux microscope with variable phase contrast optics and a Reichert camera were used with Ektachrome film (ASA 160, tungsten).

Growth Experiment. The purpose of the growth experiments was to determine a suitable range of microcapsule concentration to use in subsequent experiments. Growth was the definitive indicator of digestion and utilization of microcapsules.

1. Straight-hinge oyster larvae were grown in 250-l larval tanks with three different concentrations of gelatin-acacia microcapsules containing cod liver oil: 500, 1,600, and 5,000 microcapsules/ml. Starved larvae and larvae fed with *P. paradoxa* were used as controls for this experiment. Some gelatin-acacia microcapsules containing cod liver oil were supplemented with vitamins B_1, B_2, and B_{12}. Arbitrarily, the ratio of microcapsules without vitamins to those with vitamins was 6:1. Microcapsules were added to the tanks every day and the seawater was changed every second day. The number and size of the larvae were determined on days 3, 5, 11, 13, and 17. Larvae were concentrated (50–250/ml) for counting; the anterior to posterior length of 20 of these larvae were measured.

2. Straight-hinge larvae were cultured in 300-ml glass beakers with different concentrations (50, 100, 200, 500, 1,000, and 5,000) of microcapsules/ml. Cod liver-filled microcapsules were added every day and the seawater changed every second day. Twenty larvae were measured at 16 days.

RESULTS

Feeding and Digestion Activity

Larvae were observed to ingest and digest both gelatin-acacia and nylon-protein microcapsules. Sudan Red-stained gelatin-acacia microcapsules in the position of the stomach and digestive diverticular were observed to fade during the first 24 hours after feeding was terminated, and completely

disappeared within 48 hours. Approximately 72 hours elapsed for the larvae to completely digest the nylon-protein microcapsules. Microcapsule-fed larvae appeared healthy and vigorous throughout the test. In this feeding experiment, both types of microcapsules supported some growth. The "starved" larvae stayed in the straight-hinge stage throughout the experiment (Figure 1a) while the microcapsule-fed larvae developed to umbo stage (Figures 1b and 1c).

Growth Experiment

Growth rates of oyster larvae cultured in hatchery-size larval tanks on several diets are shown in Figure 2. Microcapsule-fed larvae grew as rapidly as those fed with the alga, *P. paradoxa,* until about day 11, and grew much better than the starved control larvae. Survival was 33% for all treatments with the exception of the 5,000-microcapsule/ml concentration which had less than 10% survival at day 13.

Some growth was evident for every concentration of microcapsules used; growth was reasonably constant above 500 microcapsules/ml (Figure 3) based on results for 16-day-old larvae grown in 300-ml beakers. Least squares analysis of length of 16-day-old larvae and capsule concentration gave a correlation coefficient of 0.72. Larvae grown in the larval tanks showed a similar trend in response to microcapsule concentrations below 2,000-microcapsule/ml (Figure 4). The reduced growth rate at 5,000 microcapsules/ml could not be explained.

DISCUSSION

Both the gelatin-acacia and nylon-protein microcapsules were acceptable to larvae of *C. virginica.* Nylon-protein microcapsules were not as digestible as the gelatin-acacia microcapsules presumably because the nylon-protein wall was formed by cross linkage between nylon and protein. The nylon-protein wall is, therefore, less susceptible to attack by digestive enzymes than the gelatin-acacia wall. Jones and Gabbot (1976) have shown that if the nylon content is decreased, the wall becomes more susceptible to proteolytic breakdown. The nylon content can be diminshed by reducing the concentration of 1,6-diaminohexane during preparation of the capsules.

It should be emphasized that these experiments were set up primarily to test the acceptability and digestibility of these two types of microcapsules; detailed consideration was not given to requirements for optimal growth. It was interesting, therefore, to find that gelatin-acacia microcapsules filled with cod liver oil were supportive of larval growth and development. Other investigators (Jones et al. 1974, Gabbot et al. 1975, Jones and Gabbot 1976, Jones et al. 1979a, Jones et al. 1979b) also reported that nylon-protein capsules containing protein, starch, and cholesterol supported growth of both the brine shrimp *Artemia,* and the Japanese oyster *Crassostrea gigas.* In our experiments, gelatin-acacia capsules contained only lipid, with the exception of the small amount of protein in the gelatin and

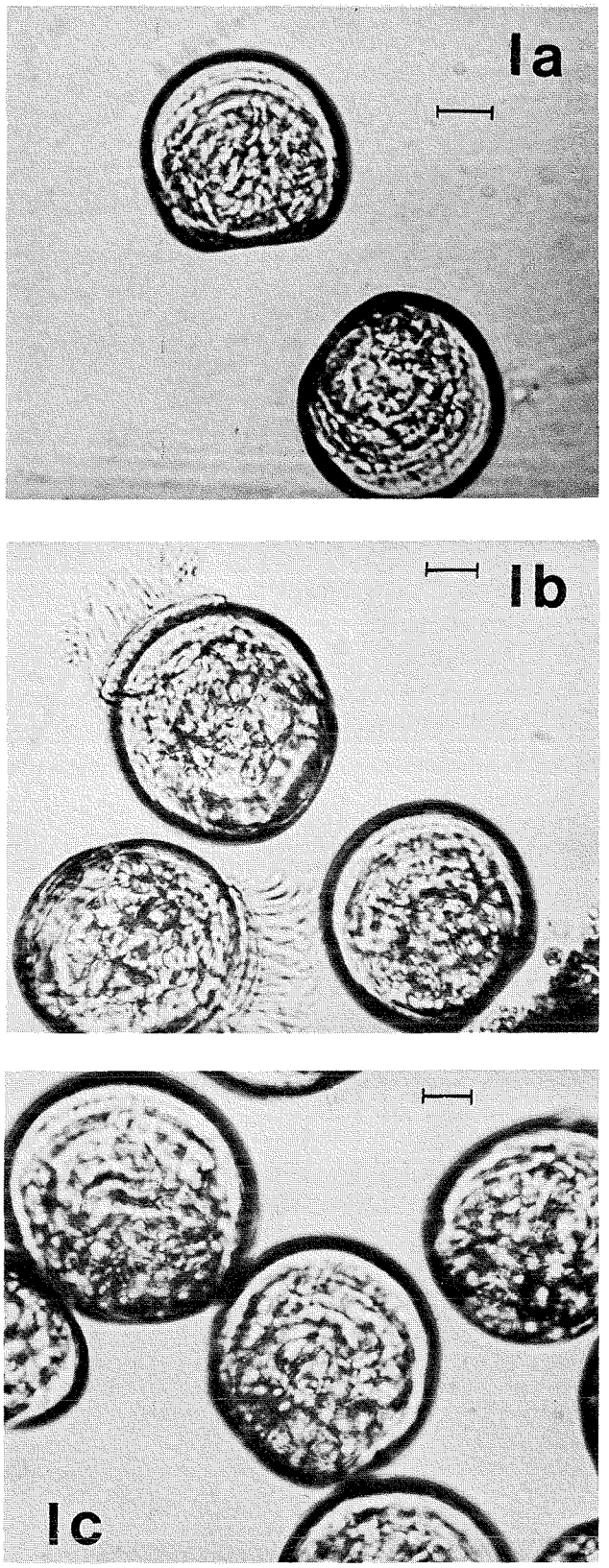

Figure 1. Photomicrographs of 4-day-old oyster larvae: (a) "starved" controls; (b) those fed with gelatin-acacia microcapsules; (c) those fed with nylon-protein microcapsules. (Note that the fed larvae progressed to the umbo stage.) Bar = 20μm.

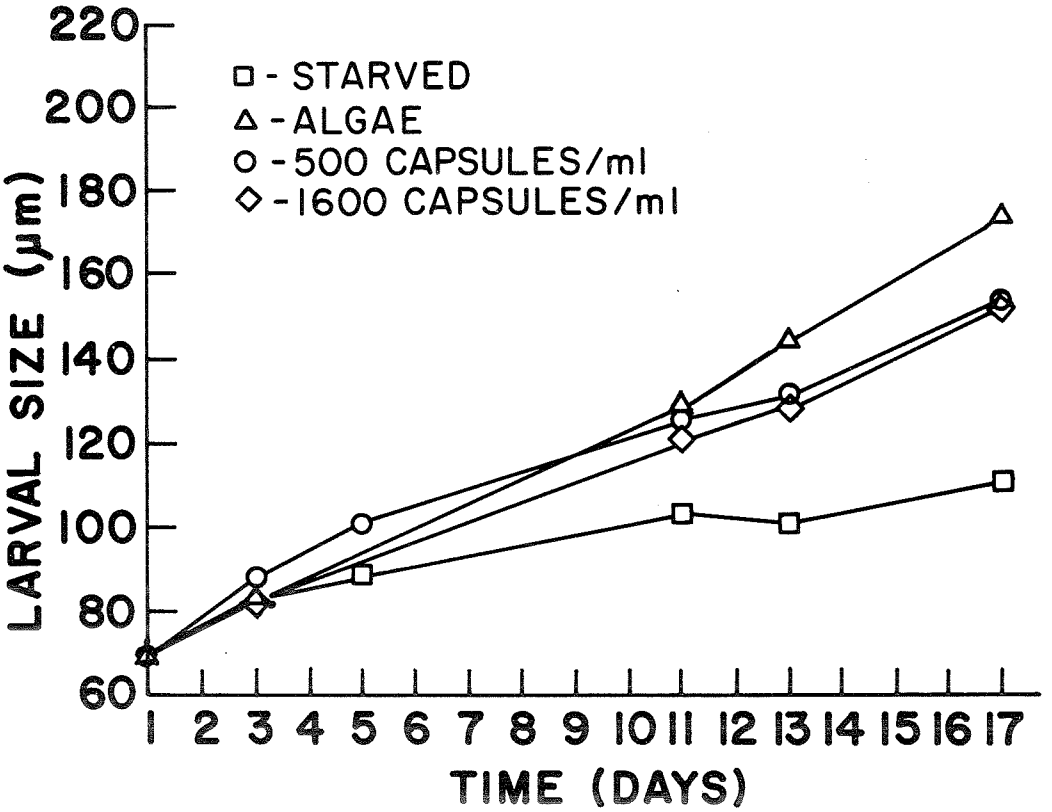

Figure 2. Growth of oyster larvae under different feeding conditions. Larvae were raised in hatchery-size tanks.

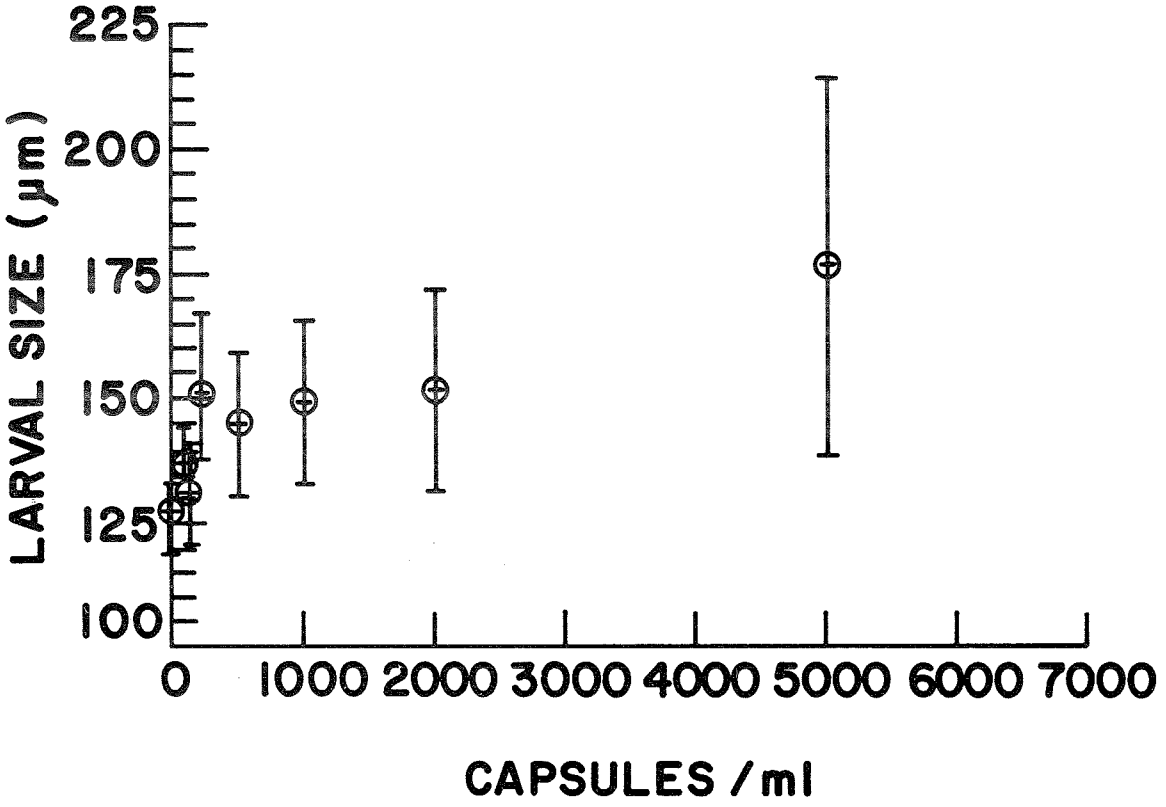

Figure 3. Size of 16-day-old larvae in 300-ml beakers versus concentration of microcapsules. (Standard deviation is indicated by the vertical bars; n = 20.)

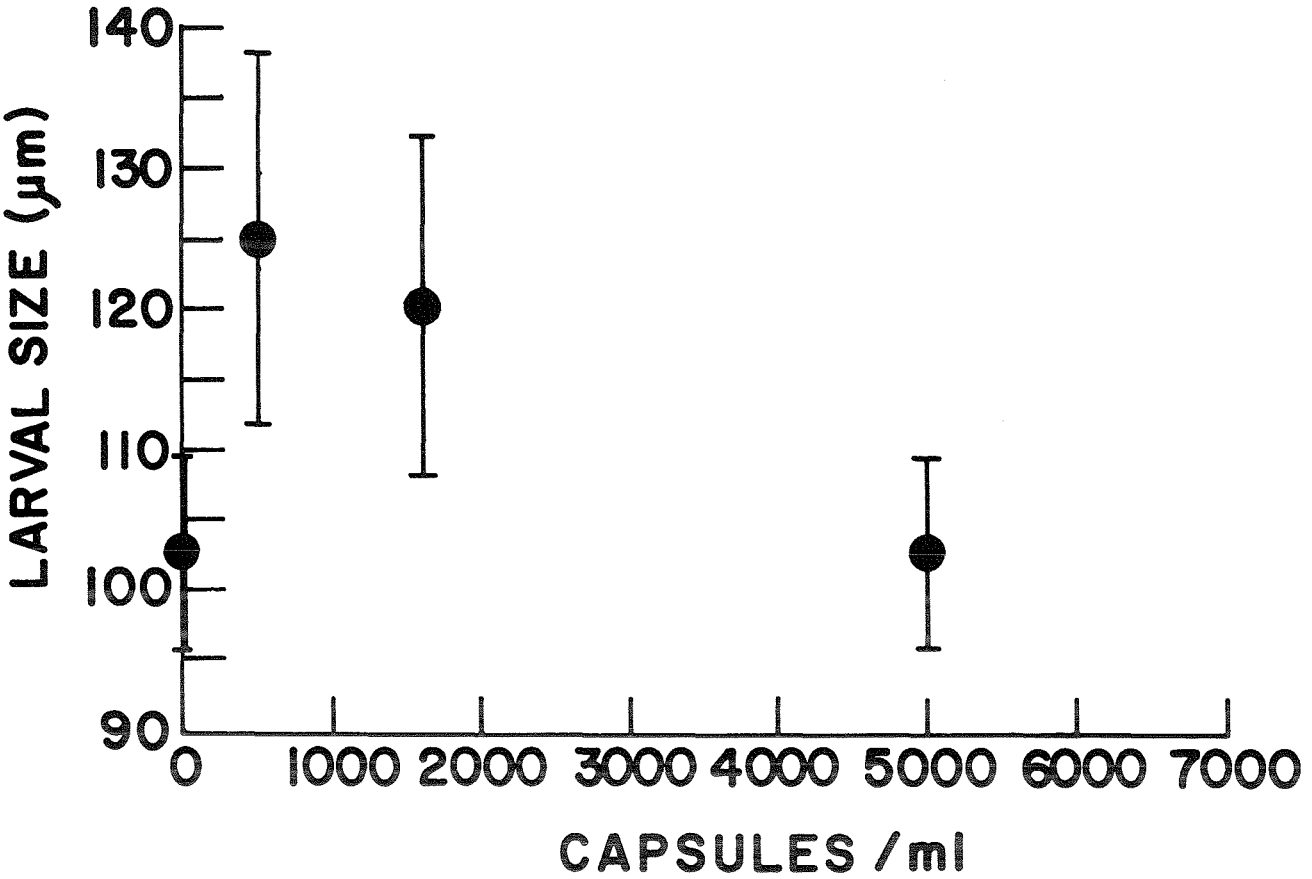

Figure 4. Size of 11-day-old larvae in hatchery-size tanks versus concentration of microcapsules. (Standard deviation is indicated by the vertical bars; n = 20.)

carbohydrate in the acacia. We anticipate better growth when optimal proportions of lipid, protein, and carbohydrate are encapsulated. There are indications that fatty acids may play a significant role in the metamorphosis and development of oyster larvae (Helm et al. 1973, Holland and Spencer 1973, Holland 1978, Waldock and Nascimento 1979, Chu and Dupuy 1980). Increasing the supplement of lipid which contains large amounts of long chain polyunsaturated fatty acids (e.g., 22:5w3 and 22:6w3) in the diet could be a promising approach.

Because it is unlikely that vitamins would be present in sea water in sufficient quantity for growth, supplements of B_1, B_2, and B_{12} were provided. Vitamin B_{12}, which is water soluble and may leach out during encapsulation, was retained in part by the gelatin-acacia capsules. It is bright red in color and the capsules with B_{12} were slightly pink.

Gelatin is very susceptible to bacterial attack and bacteria may be attached to the capsule walls. Although bacterial contamination could not have been the source of the bulk nutrients, they may have been the source of trace materials.

It is a disadvantage that the gelatin-acacia wall is permeable and the nylon-protein wall is semi-permeable to small molecules. Only water-insoluble and macromolecular components of the diet can be contained within such capsule membranes without loss. It would be ideal to produce a capsule with double walls because that type of capsule might be suitable for the encapsulation of both low-molecular weight and water-soluble components (e.g., amino acids and vitamins) as well as lipids. In this approach the aqueous solution would be encapsulated within the lipid before the second outer wall is formed.

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