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71. MICROENCAPSULATED DIET AS SUPPLEMENTAL FOOD FOR LARVAE AND SPAT OF THE PEARL OYSTER *PINCTADA FUCATA*

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ABSTRACT

The flagellate *Isochrysis galbana* was found to be the best natural food for the pearl oyster larvae. To supplement this, artificial microencapsulated diet was prepared using edible oyster oil, fish oil and Soybean lecithin with a view of enhancing the growth of pearl oyster larvae and spat. Different concentrations of edible oyster oil diet, fish oil diet and lecithin diet were tried by keeping a control wherein *I. galbana* was alone given as feed. The control with *I. galbana* gave good results. Among the artificial diets tested the edible oyster oil diet showed better results on the growth of larvae and spat while the fish oil diet promoted weight gain in the spat. The suitability of the microencapsulated diet for the larvae and spat is discussed in this paper.

INTRODUCTION

Large scale production of seed of the Indian pearl oyster *Pinctada fucata* was done in the shellfish hatchery at the Tuticorin Research Centre of Central Marine Fisheries Research Institute using the live natural food *Isochrysis galbana* (Alagarswami et al, 1983). Acceptability of other microalgae such as *Pavlova lutheri, Dicrateria* sp and *Chromulina* sp was also tested, In all these natural diets the larvae grew and set as spat between 18 and 20 days. In order to promote faster growth of larvae and early settlement, artificial encapsulated and formulated diets were prepared. Earlier reports on artificial diet as bivalve larval feed are rather limited. However, some studies were reported on the effect of dried particles of *Ulva*, *Fucus* or *Laminaria* on clam larvae which grew to metamorphosis in 13-17 days. The larvae receiving frozen *Ulva* did not grow as rapidly as those receiving live foods but reached metamorphosis in 8-13 days (Chanley and Normandin 1967). Stickney (1964) fed clam larvae with ground and strained *Zostera* leaves but the larvae did not grow on this food mixture even with the addition of antibiotics to control the bacterial population. The effect of algal and artificial diets on the growth and fatty acid composition of *Crassostrea gigas* spat was studied by Langdon and Waldock (1981). The attempts to promote larval growth on various organic materials such as yeast cereals, baby foods etc., have remained largely unsuccessful and unreported (Ukeles 1975). However, in the present study, a preliminary attempt

has been made in feeding the pearl oyster larvae and spat with artificial diet. The results of the experiments are given in this paper.

MATERIAL AND METHODS

The larvae of *P. fucata* used are from the shellfish hatchery and are from the same brood. The larvae were transferred, on the fifth day after spawning, to one litre filtered seawater in a glass beaker at 12 larvae/ml. The size at dorsoventral aspect was taken as the parameter in growth studies. The average size of larvae in the experiment varied from 81.0 μm to 91.3 μm. Weekly sample of 30 larvae in each concentration was measured. No aeration was provided during the larval phase in any of the concentrations. Water was changed on alternative days. During the experiments, which lasted for 35 days, the water temperature varied between 23.9°c to 29.0°c (av. 25.4°c); salinity from 25 6°/$_{oo}$ to 32.55°/$_{oo}$ (av. 28.34°/$_{oo}$); oxygen 3.4 to 4.2 ml/1 (av. 3.77 ml/1) and pH 7.53 to 8.20 (av. 7.85).

I. galbana was fed to the larvae at 5000 cells/larva/day in all the concentrations as the main live natural food. The size of the alga was 8 μm. In addition to this, the three gelatin encapsulated diets prepared using oyster oil diet (OOD), fish oil diet (FOD) and lecithin diet (LD) were given as supplemental feed. The size of encapsulated diets (OOD, FOD and LD) varied from 2 to 5 μm. Two concentrations, 1000 capsules/larva/day (OOD-1) and 2000 capsules/larva/day (OOD-2) in each of the three encapsulated diets were tried. A control was kept for each set wherein only *I. galbana* was given as feed.

The spat selected for the assessment of OOD was 1.5 months old and had an average size range of 1252.5 μm to 1443 3 μm, for FOD it was between 930.8 μm to 1065.0 μm and for control 1071.8 μm to 1125.8 μm. For each study 200 spat were taken in one litre glass beaker. The spat were measured weekly while water was changed on alternative days. *I. galbana* was rationed to all at 50,000 cells/spat/day. It was supplemented with OOD at 10, 20, 30, 40 and 50 thousand capsules/ spat/ day in each beaker. A duplicate was kept for these concentrations. The control beaker received oniy / *galbana*. A similar feeding regimen was also followed for FOD. Water temperature ranged between 22.7°C and 29.8°C (av. 26.0°C) during the experimental period of 37 days.

A third set of experiment was conducted using larger (3.9 months old) spat of about 2890μm in average. OOD and FOD were given at two levels—20,000 and 40,000 capsules/spat/ day. Only for FOD, in addition, 80,000 capsules/spat day was also tried. Herein the LD was not used. All the beakers received *I. galbana* at 3.3 million cells/spat/day while the control was given algae only. 50 spat were tested in each concentration. Weekly sample of 30 spat were measured and were also weighed. The water temperature recorded during the experimental period of sixteen days varied from 22,7°c to 29.8°c (av. 26.0°c).

The microparticulate diet and floculated natural diet comprising mixed phytoplankton were also tested in the pearl oyster larvae separately each in two 1 l beakers. Two beakers were also kept as control where *I. galbana* alone was given

PREPARATION OF DIETS

Capsules

Gelatin coated microcapsules were prepared from the meat of *Crassostrea madrasensis* (OOD), Cod liver oil (FOD) and Soybean lecithin (LD) using chloroform-methanol method. The solvent soluble factions were extracted from the homogenated oyster meat and were concentrated with the help of rotary vacuum evaporator. It was further concentrated by drying over nitrogen gas. 2 g diet ingredient was weighed and mixed with 80 ml of 92% gelatin solution. A jet of nitrogen was passed till the capsules were fully formed. The mixture was then placed on a water bath at 40°C and homogenised at 14,000 rpm for two minutes. Subsequently the speed was reduced to 500 rpm. The pH of the mixture was reduced to 3.9 by adding 0.01M HCl drop by drop. It caused the gelatin to coacervate around each lipid droplet The stirring was reduced to 100 rpm at 40°C for 40 minutes. Now the pH was

raised to 9.3 by dropwise addition of 1 M NaOH. The capsule suspension was added to 600 ml of distilled water at a temperature of 5°C and stored there for 1 h to harden the capsules. The solution containing microencapsulated diet was autoclaved at 115°C for 15 minutes and stored in a freezer. Everyday, after bringing a small subsample to the room temperature the diet was given to the larvae.

Carageenan microbinding diet

Artificial micropartculate diets were prepared using Carageenan and Zein as binding substances. The composition of the particulate diet is given in the Table 1.

10 g of diet ingredients was mixed well with 40 ml of distilled water. The mixture was kept on a water bath at 80°C and Carageenan was added slowly with constant stirring. The diet was cooled in a refrigerator for 30 minutes. The solid diet was cut into small pieces and a bit was tested for the binding effect. The diet was then freeze-dried, made into powder and then sieved through 20μm mesh.

Zein microbinding diet

10 g of the diet ingredient was mixed with 25 ml of Zein solution which was prepared by dissolving 1.0 g Zein in 25 ml of 60% ethylalcohol. The whole mixture was freeze-dried. powdered and sieved through 20 μm mesh

Floculated natural diet

Mass culture of mixed phytoplankton was raised in 1 t capacity tank in the open light by adding 30 l fresh seawater as inoculum. The thick green bloom of mixed phytoplankton was precipitated using KOH solution. The bloom was then concentrated to 30 l, filtered and freeze-dried. The diet was stored in refrigerator.

RESULTS

The effect of encapsulated diets such as oyster oil diet, fish oil diet and lecithin diet as supplemental feed for the pearl oyster larvae was shown in the Table 2. In all cases control gave better results. The larvae fed with *I. galbana* and OOD in the ratio 5 : 1 (OOD-1) showed a growth rate of 1.91 μm per day: In the 5 : 2 ratio (OOD-2) it was 1.77 μm and 4.11 μm in 5 : 0 ratio (control).

TABLE 1. *Diet composition of microparticulate food*

Ingredients	Carrageenan (5 g) as binding substance		Zein as binding substance	
	Diet 1	Diet 2	Diet 1	Diet 2
	(g/100 g diet)		(g/100 g diet)	
Egg yolk	5.0	5.0	5.0	5.0
Casein	19.0	20.0	19.0	20.0
Egg albumin	5.0	5.0	5.0	5.0
Soybean lecithin	1.0	1.0	1.0	1.0
Yeast extract	5.0	5.0	5.0	5.0
Cholestrol	1.0	1.0	1.0	1.0
Lactose	20 0	25.0	20.0	25.0
Oil mix	5.0	5.0	5.0	5.0
Mineral mix	5.0	5.0	5.0	5.0
Vitamin mix	3.0	3.0	3.0	3.0
Aminoacid mix	5.0	5.0	5.0	5.0
Methionine	1.0	1.0	1.0	1.0
Skin milk	25.0	19.0	25.0	19.0
Carrageenan	—	—	—	—
Zein solution	—	—	25 ml	25 ml
Water	400 ml	400 ml	—	—

TABLE 2 *Growth of larvae of* Pinctada fucata *on encapsulated diet as supplemental feed for 35 days.*

No. of cells/larva/day			Length of larvae in DVM (μm)		Growth increase	Rate of growth	Day of settlement	Percentage settlement
			Initial	Final	(μm)	per day(μm)		
I. galbana	—	OOD						
5000	—	1000	82.7	149.5	66.8	1.91	34th	1.27
5000	—	2000	81.2	143.2	62.0	1.77	34th	21.68
5000	—	Nil	84.2	228.2	144.0	4.11	27th	27.45
I. galbana	—	FOD						
5000	—	1000	81.0	135.3	54.3	1.55	34th	13.45
5000	—	2000	81.3	126.8	45.5	1.30	34th	6.15
5000	—	Nil	83.0	206.5	123.5	3.53	27th	12.5
I. galbana	—	LD						
5000	—	1000	82.7	115.0	32.3	0.92	N.O	0.017
5000	—	2000	*	—	—	—	—	—
5000	—	Nil	91.3	141.8	50.5	1.44	34th	15.95

* Total mortality
N. O. Not observed

In similar feeding combination between *I. galbana* and FOD, the rate of growth per day was 1.55 μm in FOD-1; 1.30 μm in FOD-2 and 3.53 μm in the control. With regard to the lecithin diet the result was very poor being 0.92 μm in LD-1 and 1.44 μm in the control. The larvae fed with LD-2 did not survive.

The acceptability of encapsulated diet in the larvae was assessed on the basis of growth and percentage of spat settlement. The settlement of spat occurred on 27th day in the controls and 34th day in other concentrations. The percentage of settlement was 1.27% in OOD-1, 21.68% in OOD-2 and 27.45% in OOD-control. In FOD feeding the percentage of settlement was 13.45% in FOD-1, 6.15% in FOD-2 and 12.5% in FOD-control while in LD-1 it was 0.017%; nil in LD-2 and 15.95% in LD-control.

Few larvae were found dead during the experiment in OOD-1, OOD-2, FOD-1 and FOD-2. All the larvae fed with LD-2 died by the second day. Fresh set of larvae was replaced in LD-2 and they too met with a similar fate. Pseudofaeces were seen in LD concentrations. As a result of mortality in different concentrations the development of ciliates occurred in the respective media. Mortality has also occurred in the controls but in less numbers. Heavy mortality was recorded in OOD-1 and LD-1 after 30 days.

The spat in the range of 1252.5μm to 1443.3 μm, when supplemented with 10, 20, 30, 40 and 50 thousand capsules of OOD to the 50 thousand cells of natural diet *I. galbana* per spat per day, the growth rate was higher in the concentrations than in the control. The growth was 17.23 μm, 15.25 μm, 12.05 μm, 19.08 μm and 16.58 μm per day in the respective concentrations and 11.44 μm in the control. It worked out to 50.6%, 33.3%, 5.33%, 66.78% and 44.93% based on the control.

The spat which ranged between 730.8 μm and 1065.0 μm showed a growth rate of 9.87 μm, 7.75 μm, 7.36 μm, 9.1 μm and 12-09 μm per day when fed with FOD in the above similar concentrations and 10.2 μm per day in the control. In contrast to OOD, the growth rate in all concentrations of FOD was less than the control except the one concentration (5000 capsules of FOD and 50,000 cells of *I. galbana*). Based on the growth rate in the control the fall was to a degree of 3.2%, 24.0%, 27.8% and 1.08% in the concentrations referred to above.

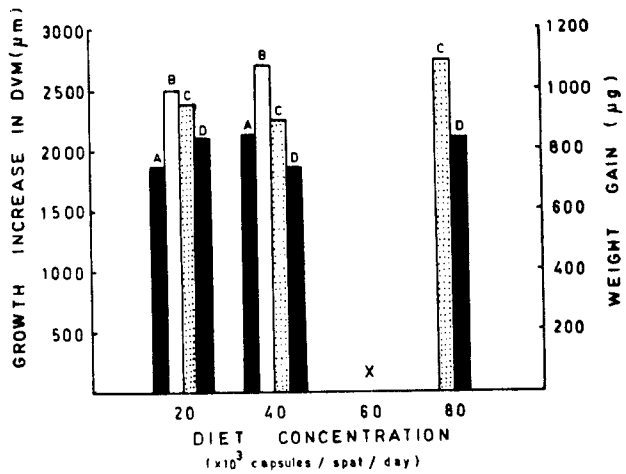

Fig. 1. Differences in the growth of pearl oyster spat fed with *I. galbana* and supplemented with oyster oil diet (OOD) and fish oil diet (FOD). A. OOD — control; B. *I. galbana* and OOD; C. *I. galbana* and FOD and D. FOD-control.

The effect of OOD and FOD on the growth of spat is shown in Fig, 1.

It is significant to note that the spat in the range 2800 μm showed a growth rate of 50μm and 57 μm per day in the concentration of 20 and 40 thousand capsules of OOD and 50 μm, 48μm and 58 μm per day in the concentrations of 20, 40 and 80 thousand capsules of FOD. In the control it was 48 μm per day. The growth was almost similar in all the concentrations. The growth and weight increase in respect of OOD and FOD at different concentrations is given in the Fig. 2.

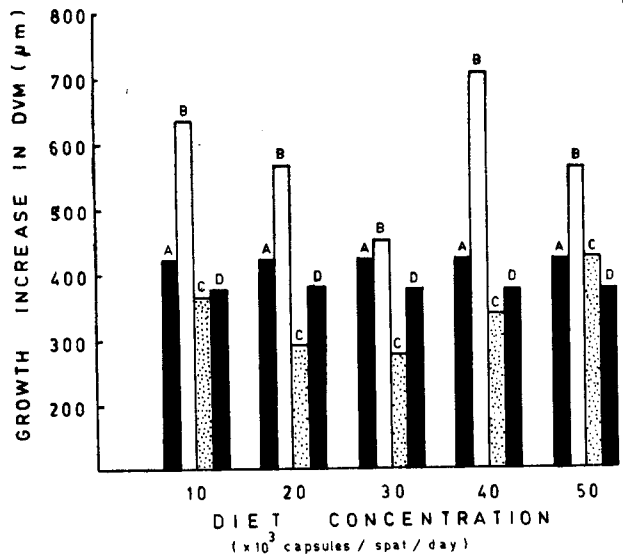

Fig. 2. Differences in growth increase and weight gain with oyster oil diet (OOD) and fish oil diet (FOD) as supplemental feed. A. Weight gain with OOD; B. Growth increase with OOD; C. Growth increase with FOD and D. Weight gain with FOD.

The pearl oyster larvae fed with artificial microparticulate diets survived only two days. The diets, on supplying to the medium, sunk to the bottom and are not available to the swimming larvae. Moreover separation of these particles from the larvae was found difficult. Accumulation of such particles in the medium resulted in the development of ciliates. Similar effect was also observed when floculated mixed phytoplankton was added to the medium. The addition of this food increased pH of the medium as it had KOH which was used as floculation agent. It is known that addition of food material other than live leads to the contamination of medium.

DISCUSSION

Early literature concerning the value of non-algal foods such as bacteria, detritus and dissolved organic compounds has been reviewed by Ukeles (1971). The results of these studies are inconclusive and there is little quantitative information to support that the filter feeding bivalve molluscs can be supported entirely with non-algal feed. Knowledge on artificial diet as supplemental feed for bivalve larvae was limited. Carriker (1956) reared clam larvae on an extract of cereal and concluded that good growth was due to the bacterial population appearing as a result of rich cereal extract. The improved growth rate of the larvae was related to an increase in a selected group of bacteria (Hidu and Tubiash 1963). Clam larvae when fed dried *particles* of *Ulva*, *Fucus* or *Laminaria* grew to metamorphosis in 13-17 days. Larvae reared with frozen *Ulva* did not grow as rapidly as those receiving live foods (Chanley and Normandin 1967). Loosanoff and Davis (1963) reported that dried, ground seaweed *Scenedesmus* sp when fed to clam larvae the growth was as effective as the one fed with best live naked flagellates. Stickney (1964) showed that the clam larvae fed with ground and strained *Zostera* leaves did not grow even with the addition of antibiotics to control the bacterial population. In the present study on the pearl oyster larval nutrition, the natural diet *I. galbana* gave good results than the encapsulated diets. The oyster oil diet has yielded better results than fish oil and lecithin.

It indicated that the diet prepared from the allied (molluscan) group promoted better growth and settlement than fish oil and lecithin diets. When compared to the natural diet the results are not encouraging. Yet it is considered to be a significant step that the larvae could grow, metamorphose and set as spat on feeding these diets.

Interest in the nutritional requirements of juveniles and adult bivalves has increased considerably in recent years. But no nutritionally adequate formulated diet for bivalves has yet been developed. However, Langdon and Waldock (1981) demonstrated that increasing the lipid content of the diet with triolein capsules did not enhance the growth of spat fed on *Tetraselmis suecica* or *Dunaliella tertiolecta* but the addition of encapsulated oyster lipid extract to the diets increased the growth. Castell and Trider (1974) have reported that the adult oyster *Crassostrea virginica* when fed with artificial diet showed lesser growth rate compared to those fed with natural diet. Trider and Castell (1980) reported that hydrogenated coconut oil fed oysters showed little weight gain after 30 weeks. The diets with higher levels of fatty acids produced significantly greater weights and the diets containing cod liver oil, ethyl esters, either sterol free or supplemental with 1% cholesterol produced significantly poorer weights than natural food. In the case of pearl oyster spat the growth was better in OOD than FOD but in respect of weight gain remarkable increase in FOD fed spat was worth noting. It can be seen that OOD has promoted length increase whereas FOD weight gain. Negligible mortality of spat on these diets proved their acceptability. Further work on these lines would perhaps help in developing suitable food in respect of fast growth and high survival rate of pearl oyster spat.

Valuable contributions have been made in the processed natural diet either as major source of food or as supplemental one. Lyophilised cultures of the flagellates, *Dunaliella euchlora* and *Isochrysis galbana* fed to clam larvae produced survival and growth comparable to that obtained when clams were fed live algae. The larvae of the American oyster *Crassostrea virginica* failed to grow on lyophilised preparations of *I. galbana* (Hidu and Ukeles, 1962). Spray-dried *Chlorella* cultures from Japan, a culture of *Monochrysis luther* vacum dried in manitol and lyophilised *I. galbana* gave similar results with larvae of European oyster *Ostrea edulis* (Walne 1974). Loosanoff and Davis (1963) showed that the clam larvae fed freeze-dried *I. galbana* grew as rapidly as larvae fed live cells of the same alga. In the present study the pearl oyster larvae fed with floculated, freeze-dried samples of mixed phytoplankton did not yield results.

Failure of microparticulate diet as artificial feed for pearl oyster larvae and spat way due to the following reasons: 1) It was found difficult to grind the microparticulate diet into particles small enough to be ingested by the larvae or spat; ii) The particles quickly settled on the bottom thus becoming unavailable to swimming larvae; iii) Separation of these particles from the larvae was found difficult and iv) Finally because of rapid decomposition of microparticulate diet, the larval cultures became fouled and the bacterial flora and ciliates developed

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