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70. MICROENCAPSULATED DIET FOR LARVAE AND SPAT OF *CRASSOSTREA MADRASENSIS*

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ABSTRACT

Edible oyster larvae and spat were fed with *Isochrysis galbana* supplemented with microencapsulated diet prepared from oyster, clam or fish oil extracts. In the experiments conducted with oyster larvae, spat setting was higher in the larvae fed with algal diet supplemented with oyster oil extract encapsulated diet than those fed with algal diet. Better growth and more weight increase was observed among the spal fed with algal diet supplemented with microencapsulated diet containing oyster oil and fish oil, compared to that in oyster spat fed with algal diet alone.

INTRODUCTION

Seed production of edible bivalves is one of the prime requisites for carrying out large-scale farming. Study on the mass production of oyster seed through hatchery system was initiated by the Central Marine Fisheries Research Institute and successful production of seed oysters has been achieved at the Shellfish hatchery at Tuticorin (Nayar et al 1984). In the larval rearing and rearing early spat in the hatchery, cultures of microalgae such as *Isochrysis galbana* and *Pavlova lutheri* are maintained. Though mass culturing feed algae is cumbersome and has many drawbacks over suitable formula feed, at present no suitable feed for molluscan larvae is available. Microparticulated diets such as Carrageenan microbinding diet and Zein microcoated diet which have been successfully used as alternate diet in Prawn larval rearing (Kanazawa et al 1982), was tried for the edible oyster larvae and spat. Since the particle when soaked in water swells up and enlarges in size it was found to be too large for the molluscan larvae and so was not useful for feeding larvae and spat. As the oesophageal diameter of molluscan veliger is about 5 μm, the optimum particle size for the molluscan larval feed is μm.

Keeping in view the usefulness of microencapsulated diets in the larval rearing of marine prawns (Jones et al 1979), these diets were prepared in the present study from edible oyster extract, clam extract and fish oil and were tried alongwith regular algal diet. Helm et al (1973) and Holland and Spencer 1973) demonstrated the importance of lipid in the early development of oysters.

MATERIAL AND METHODS

Preparation of Capsule

Gelatin capsules were prepared by the method given by Langdon and Waldock (1981). At 40°C in an atmosphere of nitrogen 1 ml of lipid was emulsified in dark for 2 minutes in 40 ml of 2% W/V aquous gelatin solution using an homgenizer maximum speed (14,000 rpm). The emulsion was then transferred to a flask and stirred at 500 rpm for 1 min. The pH of the mixture was reduced to 3.9 by adding 0.01 M-HCl, causing a gelatin wall to form around each lipid droplet. The rate of stirring was then reduced to 100 rpm and the mixture was kept at 40°C, for 40 min. After stirring, the pH was raised slowly to 9.3 by adding 1M-NaOH dropwise. This capsule suspension was poured into 300 ml distilled water at 5°C for at least 1h to harden the capsule walls. The capsules then were autoclaved at 115°C for 15 min without any adverse effect on wall stability. The suspensions were stored at 5°C until required. The mean diameter of the capsules was 3μm.

Edible oyster and clam extracts were prepared by using Chlorofom Methanol mixture (2:1) after Bligh & Dyer method. The oyster and clam meats were collected and homgenised using high speed blender. The homogenate was treated with large quantity of solvent mixture and the soluble fraction was concentrated by

rotary vaccum evaporation. The final concentration was carried out by drying the concentrate over nitrogen gas. The concentrated extract was used as the diet ingredient for the preparation of microencapsulated diets. Cod liver oil (available in the market under the trade name Seven Seas) was directly used as the fish oil diet. The diets were prepared and stored in the refrigerator under nitrogen for a number of days.

Edible oyster larvae of 70μm belonging to the same brood were used in the study. They were fed with *Isochrysis galbana* supplemented with one of the microencapsualted diets prepared from edible oyster extract (OOD) and clam extract (COD). (Larvae were reared in 3 l glass beakers in a concentration of 4 larvae per ml. Filtered seawater was changed daily.) Larvae were fed with *I. galbana* @ 5000 cells/larva/day and supplementary diets OOD and COD at two levels viz, 10,000 capsules and 20,000 capsules per larva per day (Table 1). Experiments were conducted in triplicate and the rate of settlement of spat between the diet groups was compared with the control which were fed only with *I. galbana*.

For the spat growth experiments spat of 1 to 2 weeks old of same brood were starved for 24 h before the experiment, cleaned, blotted dry and weighed. Experiments were conducted in triplicate for a particular diet with 50 or 100 spat kept in a 5 l beaker. Filtered seawater was changed daily. *I. galbana* @ 20,000 cells/ spat and supplementary diets of OOD, COD and FOD/20,000 to 60,000 capsules were given daily.

RESULTS

Larval feeding experiments

The number and percentage of spat settled in the two experiments are given in Table 1. In experiment No. 1, one set of oyster larvae was fed with *I. galbana* alone for 25 days and average settlement was 5.9%. In the larvae fed with *I. galbana* and 10% OOD the percentage of settlement was 3.5% and for 20% OOD, the percentage of spat settled was 8.4. The clam lipid extract diet supplemented with *I. galbana* fed larvae, the spat settlement was 1.8%.

In the second and third experiment also the percentage of spat settled in the larvae fed with *I. galbana* supplemented with 10% and 20% OOD was found to be more than for the larvae fed with *Isochrysis galbana* alone or *I. galbana* supplemented with 10% COD.

Spat growth experiments

In Table 2, the weight increase of spat fed with *I. galbana* and microencapsulated diets supplemented with *I. galbana* are given for the three experiments conducted.

In the Experiment I, the weight increase for the spat fed with *I. galbana* supplemented with 20,000 cells of OOD/spat was 97% and with

TABLE 1. *The percentage of larvae* (C. madrasensis) *setting, fed with artificial supplimental diet.*

Expt. 1 22.6.84 to 9-7-84	No. of spat settled	% of settlement.
I. galbana + OOD 10%	286	3.5
I. galbana + OOD 20%	674	8.4
I. galbana + COD 10%	149	1.8
I. galbana only	475	5.9
Expt. 2 25.9.84		
I. galbana + OOD 10%	220	1.8
I. galbana + OOD 20%	136	1.1
I. galbana + COD 10%	75.6	0.6
I. galbana only.	0	0

OOD = Oyster oil diet; COD = Clam oil diet

TABLE 2. *The growth rate of the spat of* C. madrasensis *fed with supplimentary artificial diet.*

Initial wet wt of spat : 0.092 gm Mean length of spat : 1.16 mm Duration of the expt : 19 days	increase in in length (mm)	Wet weight increase (%)
Expt. 1. 23.12.83 to 11.1.84		
1. *I. galbana* + OOD (20,000 cells/spat)	1.13	97.0
2. *I. galbana* + OOD (40,000 ,,)	1.03	122.0
3. ,, + FOD (20,000 ,,)	1.06	85.0
4. ,, + FOD (40,000 ,,)	1.16	93.0
5. ,, + FOD (80,000 ,,)	1.02	86.0
6. *I galbana* only	0.67	69.0
Expt. 2.		
Duration 24 days. 7.1.84 to 30.1.84		
1. *I. galbana* + OOD (20,000 ,,)	—	133.0
2. ,, + OOD (40,000 ,,)	—	140.0
3. *I, galbana* + FOD (20,000 ,.)	—	100.0
4. ,, + FOD (40,000 ,,)	—	85.9
5. ,, + FOD (60,000 ,,)	—	97.6
6. *I. galbana* only	—	121.0

Expt. 3.				
23.4.84 to 22.5.84				
Initial length : 2 4 (mm)				
1A *I. galbana* + 20% (capsules)	0.134	0.396	0.262	195.0
1B	0.154	0.460	0.306	198.0
2A *I. galbana* + 40% OOD ,,	0.134	0.356	0.216	161.0
2B	0.155	0.350	0.195	125.0
3A *I. galbana* + 10% COD ,,	0.200	0.587	0.387	193.5
3B	0.162	0.402	0.240	148.1
4A *I. galbana* + 10% FOD ,,	0.197	0.616	0.419	212.0
4B	0.163	0.586	0.423	259.0
5A *I galbana* only —	0.120	0.341	0.229	184.0
5B	0.134	0.381	0.247	184.0

OOD = Edible oyster oil diet; COD = Clam oil diet; FOD = Fish oil diet (cod liver oil)

40,000 cells of OOD/spat was 122%. The Fish oil supplemented at the rate of 20,000, 40,000 and 80,000 cells/spat along with *I. galbana* registered an increase of weight 85%, 93% and 86% respectively. The control spat fed with *I. galbana* alone had weight increase of 68%. In the second experiment also, the spat fed with the micro algae supplemented with OOD had achieved higher percentage of weight increase than those fed with *I. galbana* alone and the other set of spat fed with *I galbana* supplemented with fish extract.

In the third experiment one set of spat was fed with *I galbana* supplemented with 10% COD and the weitht increase was 170%. The control sets, the percentage of weight increase was 184%. But the spat fed with algal diet supplemented with 20% OOD registered a weight increase of 198%.

DISCUSSION

The algal diets used in bivalve larval and spat rearing are varied in their food quality. Walne (1970) has given the food value of various micro algae used as feed for bivalve larvae. Trider and Castell (1980) indicated that polyunsaturated fatty acids are essential to oysters for growth and maintenance. In the hatchery experiments larvae fed with *Dunaliella salina* have not grown well indicating that the deficiency of 22:6W3 as a growth limiting factor as reported by Langdon and Waldock (1981).

Further it was demonstrated by Langdon and Waldock (1981) that the mixture of 20:5W3 (ecosa pentaenoic acid) and 22:6W3 (docosa hexfaenoic acid) support better growth than individual acids. The growth of *C. gigas* was enhanced by adding supplements of either solvent extract of oyster high in 20:5W3 and 22:6W3. The experiments in the larval rearing supplemented with OOD showed more spat settlement percentage than the larvae fed with algal diet alone and solvent extract, clam extract diet supplemented with *I. galbana* (Table 1). In spat growth experiments also, the seed oysters fed with OOD registered more weight increase than those fed with Fish oil diet and clam extract, as observed by Langdon and Waldock (1981) the improved growth in oyster spat when oyster lipid extract capsulated diet of 22:6W3 along with *D. tertiolecta* or *I. suecica*.

Of the OOD, FOD and COD microencapsulated diets OOD yielded better spat settlement, improved growth of spat indicates that the essential fatty acid of linolenic (W3) may be more than linoleic (W6) type fatty acids. Further studies need be carried out on the quality and quantity of the essential fatty acids in the capsules containing solvent extracts of oyster, clam and fish oil and also the fatty acid composition of the spat experimented with the above mentioned diets.

In hatcheries, one or two cultured algal species are being used as diet for larvae and spat. These algae in laboratory culture conditions may be unable to produce long-chain fatty acids of the W3 which is essential for the growth of bivalve larvae and spat. So further study is needed on the amount essential fatty acids composition in these algae so as to supplement with the lipid extracts and to obtain better growth of seed oysters.

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