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Studies on the nutritional value of a new type of microencapsulated diet for Pacific oyster (*Crassostrea gigas* Thunberg, 1793) larvae*

by

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INTRODUCTION

During the last century there was a dramatic decline in the self-perpetuating *Ostrea edulis* (LINNAEUS, 1758) beds around the major European oyster-producer countries, due mainly to over-fishing and bad husbandry, pollution and the effect of naturally occuring and introduced pests (GERDES, 1983). The Pacific or Japanese oyster *Crassostrea gigas* is progressively becoming a highly interesting species for European hatcheries and on-growing farms, due mainly to its high growth rate and resistence to pests (SPENCER, personal communication).

The practice of seed production in hatcheries for distribution to farmers is nowadays considered as essential to maintain and increase the production of oyster and other bivalves.

Suitability of a phytoplankton species used for feeding cultured bivalves is usually assessed in terms of biochemical composition, digestibility, toxicity and cell size. Much work has been done to obtain the most appropriate algal diet for each commercially cultured bivalve species. The results are sometimes contradictory mainly because the food value of these algal species is not constant and varies with origin and culture conditions (DAVIS, 1950; LOOSANOFF & DAVIS, 1963; WALNE, 1965; WEEB & CHU, 1981; HELM & LAING, 1986; LAING & MILLICAN, 1986).

From the aquaculture point of view, suitable algal diets are known, with the major bottleneck to commercialization of intensive bivalve culture systems lying in

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the inability to economically culture massive quantities of suitable algal species (URBAN & LANGDON, 1984).

These factors have promoted a search for non-algal food materials which both support satisfactory growth and provide a useful tool in order to assess the nutritional requirements of suspension-feeders.

The use of microencapsulated foods for aquatic filter-feeders was first described by JONES, MUNFORD & GABBOTT (1974), and there have been many attempts to develop satisfactory artificial diets for marine bivalves (CASTELL & TRIDER, 1974; LANGDON & BOLTON, 1984; LANGDON & SEIGFRIED, 1984).

Some preliminary information on the nutritional requirements of bivalves has been achieved by feeding microcapsules containing specific dietary supplements (LANGDON & WALDOCK, 1981), but the development of a complete artificial diet has yet to be achieved.

This work was designed to evaluate the growth of Pacific oyster larvae when fed a new type of microencapsulated diet produced by FRIPPAK FEEDS, Basingstoke, England. The artificial diet was compared with the diatom *Chaetoceros calcitrans*, which is an alga of proved high food value for bivalve larvae (LAING, personal communication).

In these trials, oyster larvae were reared under controlled conditions in 3 litre beakers and 40 litre bins, using larvae from the same batch for each treatment in each experiment, hatched at the laboratory.

As well as nutritional value, the microencapsulated diet was assessed in terms of biochemical composition and settlement rate. As the larvae developed, measurements of larval shell length, dry weight, ash-free dry weight, lipid, carbohydrate, and protein were made.

MATERIALS AND METHODS

Larval rearing systems

All experiments were carried out in a constant temperature room at 25 °C. For the experiments two types of culture container were used:

- 3 litre glass beakers (Trials 2, 3)
- 40 litre polyethylene bins (Trial 1).

The larvae were reared in seawater filtered through a diatomaceous earth filter (British Filters Ltd., Mod. PF 30B) to remove particles > 2 µm in size. The sea water was also ultraviolet light treated to sterilize it. Salinity was adjusted to 25 ppt with filtered fresh water. All vessels were gently aerated in order to keep the algae and food particles in suspension, and complete water changes and cleaning of all bins and beakers were made three times each week.

Supply of larvae

Eggs and sperm were obtained from broodstock conditioned at the laboratory for 4-6 weeks at 24 °C. The oysters were artificially spawned by opening mature individuals and pipetting eggs and sperm from the gonads into separate beakers, one for each animal. Fertilization was effected by mixing 2-5 ml of a dense sperm suspension in 3 litre beakers containing an even suspension of eggs. After one hour, the development of the embryos was checked and they were transferred to 125 litre incubating bins, filled with aerated sea water treated as described above.

The D-larva stage was reached within 24 hours of fertilization. After estimates of larval numbers, the experimental vessels were stocked at an initial density of 300-350 $\times$ 10^3 larvae per bin, and 10000 larvae per beaker.

Algal culture

Chaetoceros calcitrans, used as the control diet, was cultured at 20-22 °C in non-axenic, 3 litre batch cultures in medium prepared from autoclaved sea water. The light intensity measured at the culture surface was 12500 lux, and the culture was bubbled with air supplemented with 0.5 % carbon dioxide. The diameter of *C. calcitrans* cells was in the range of 2.5-3 µm diameter.

Samples of the algal diets fed to the containers were taken for estimation of ash-free dry weight and biochemistry analysis. The concentration of algal cells in the tanks before and immediately after feeding each day was determined using an electronic particle counter (Coulter, Mod. ZM). From these values the number of algal cells cleared from suspension per day were calculated.

Artificial diet

The artificial diet tested was prepared by FRIPPAK FEEDS, Basingstoke, England, and supplied as a freeze-dried powder (UK patent n.° 2040863 B and 2103568 B).

The amount added every day to the rearing containers was in proportion to the algal diet on a dry weight basis. The dry powder was rehydrated by adding tap water, blending for 2-3 min, then it was kept at 4 °C for at least one hour before use.

The artificial diet was added each day in two equal portions, one in the morning and the other 6-8 hours later. Mixed live food/artificial diet treatments were also tested.

Samples of the artificial diet were taken for estimation of particle size and number using an electronic particle counter as described above. An estimation was also made of the settling rate of both capsules and the algal diet, using 40 litre bins without larvae. Ash-free dry weight determinations and biochemical analyses were also made.

Experimental treatments

Trial 1 (40 litre bins)

In this trial, larvae were grown for 18 days from the D-larva stage (Initial average size: 80.00 µm). Feeding was started on the day of stocking and consisted of the control algal diet and mixed live food/artificial diets:

(i) *C. calcitrans*, 125 cells · μl^{-1} (Control)
(ii) 85 % *C. calcitrans* + 15 % microcapsules
(iii) 65 % *C. calcitrans* + 35 % microcapsules
(iv) 50 % *C. calcitrans* + 50 % microcapsules
(v) Microcapsules
(vi) Starved control

Biochemical composition (lipid, carbohydrate, protein), dry weights, and ash-free dry weights of larvae were determined at day 1 and at the end of the experiment. At each water change, larval number and mean shell length were determined.

Trial 2 (2 litre beakers)

In this trial, larvae were grown for 6 days from the D-larva stage. Five treatments, each in duplicate, were tested. Feeding was started on the day of stocking and consisted of control algal diet and four artificial diet treatments:

(i) *C. calcitrans*, 125 cells · μl^{-1} (Control)
(ii) Microcaps. [7 mg · d^{-1} ≡ 125 cells · μl^{-1} (dry wt) algae]
(iii) Microcaps. [3.5 mg · d^{-1} ≡ 62.5 cells · μl^{-1} (dry wt) algae]
(iv) Microcaps. [14 mg · d^{-1} ≡ 250 cells · μl^{-1} (dry wt) algae]
(v) Microcaps. [3.5 mg · d^{-1} × 2 ≡ 125 cells · μl^{-1} (dry wt) algae]

Cell clearance rates were estimated daily. Larval size and numbers were determined initially, at each water change and at the end of the experiment.

Trial 3 (3 litre beakers)

In this trial, larvae were grown for 15 days from the D-larva stage. Feeding was started on the day of stocking and all treatments were fed the control algal diet initially. When larvae reached 105.70 µm, 163.30 µm, and 237.76 µm shell length, they were fed the control algal diet, the artificial diet and unfed, each in duplicate.

Cell clearance rates were estimated daily and estimates of larval performance as in Trial 2.

Analytical methods

The methods used for lipid, carbohydrate and protein determination were those described by HOLLAND & GABBOTT (1971). Dry weights were estimated by drying larvae/diets samples for 48 h at 60 °C and ash-free dry weights by ashing dry larvae/diets samples in a Gallen Kamp muffle furnace at 450 °C for 4 hours. Both weights were calculated on a per larva basis and per million algal cell basis.

RESULTS

Diets

Rehydrated capsules were less than 20 µm diameter, with about 91 % in the range 2.5-5 µm diameter.

In 40 litre bins operated without larvae, 74 % of the 2.5-5 µm diameter capsules remained in suspension after 5 h. Nearly all capsules settled out of suspension by the end of a 30 h period. The algal cell concentration increased slightly after 30 h, as is shown in Table I.

The biochemical composition of algae and microcapsules used in trials 1-3 is shown in Table II.

Oyster larvae

The effect of different treatments in Trial 1 on growth of larvae is shown in figure 1, and the regression equations corresponding to Trial 1 are listed in Table III. The effect of different treatments in Trial 2 and 3 on growth of larvae is shown in Tables IV and V respectively.

TABLE I

Settling rate of microcapsules and the algal diet at different time periods in 40 litre bins

Time (hours)	*Particle number in suspension* × μl^{-1}			
	Algal diet (control)		*Microcapsules*	
Initial	111.12	111.65	23.94	22.25
1	110.14	111.70	21.67	21.94
3	112.15	112.94	20.02	16.37
5	112.35	112.40	18.36	14.22
20	118.12	118.14	9.83	6.93
24	120.05	120.69	9.35	6.34
30	130.23	127.26	9.00	7.00

TABLE II

Biochemical composition of algae and microcapsules used in trials 1-3. (Mean values of 3 observations)

	$\mu g \times 10^6$ cells^{-1}		Lipid		Protein		Carbohydrate	
	Dry wt.	Ash-free dry wt.	$\mu g \cdot 10^6$ cells^{-1}	% of ash free dry wt.	$\mu g \cdot 10^6$ cells^{-1}	% of ash free dry wt.	$\mu g \cdot 10^6$ cells^{-1}	% of ash free dry wt.
C. calc.	4.701	4.198	0.563	13.41	3.284	78.22	0.351	8.36
Microc. (w/w)		80 %		34.20		62.18		3.62

The results from analysis of variance (ANOVAR) on growth in relation to treatment showed that in all three trials there were significant differences in growth between treatments ($p < 0.001$). Analysis of Least Significant Difference (L.S.D.) showed that the control algal diet, *C. calcitrans*, supported significantly better growth than the single microcapsules diet in Trial 1 ($p < 0.001$). In Trial 2, the performance of larvae fed the control algal diet and the same proportion of artificial diet did not show a significant difference ($p < 0.3$), but all larvae fed the artificial diets failed to survive beyond day 4.

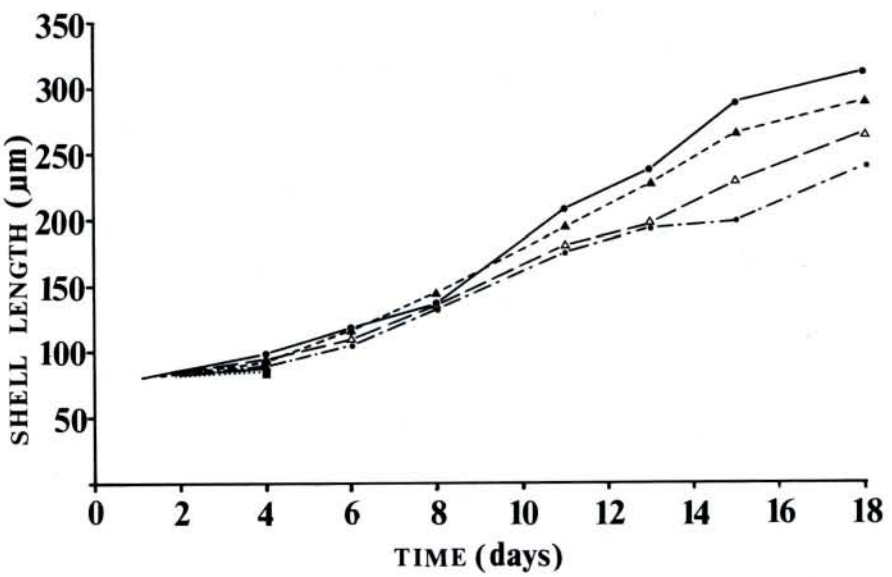

FIG. 1. – Effect of different treatments on growth of *C. gigas* larvae in trial 1. (●——●, *C. calc.*; ▲– – –▲, 85 % *C. calc.* + 15 % Microc.; △— —△, 65 % *C. calc.* + 35 % Microc.; ●— · — ·—●, 50 % *C. calc.* + 50 % Microc.; ◇— · · —◇, Microcapsules; ■ · · · · ■, Starved).

TABLE III

Regression equations of figure 1. (X = day; Y = shell length)

Diet	*Intercept (A)*	*Slope (B)*	*r*	*df*	*Level of significance*	*Type of relationship*
C. calc.	4.28	0.0877	0.981	7	$p < 0.001$	$Y = Ae^x$
85 % *C. calc.*	4.28	0.0833	0.980	7	$p < 0.001$	$Y = Ae^x$
65 % *C. calc.*	4.29	0.0749	0.989	7	$p < 0.001$	$Y = Ae^x$
50 % *C. calc.*	4.29	0.0696	0.971	7	$p < 0.001$	$Y = Ae^x$

TABLE IV

Effect of different food treatments on growth of *C. gigas* larvae in trial 2

Initial size (µm)	*Size (µm) at the end of the experiment (Day 4)*				
	C. calc	*Microc. (7 mg · d^{-1})*	*Microc. (3.5 mg · d^{-1})*	*Microc. (14 mg · d^{-1})*	*Microc. (7×2 mg · d^{-1})*
74.20	78.67	78.69	76.60	75.66	74.30

TABLE V

Effect of different food treatments on growth of *C. gigas* larvae in trial 3

Initial size (µm)	*Size at the end of the experiment (µm)*			
	Algal diet	*Microc. diet*	*Starved*	*Time (days)*
105.70	124.45	110.83	112.39	2
163.30	249.67	241.06	220.24	4
237.76	298.65	259.36	256.96	5

In Trial 1, when the algal diet was substituted with 15 % and 35 % microcapsules by weight, the growth of the larvae did not show a significant difference from the larvae fed the control algal diet at day 4 of treatment, but at day 18 there was significant difference between all treatments ($p < 0.001$).

The slopes corresponding to the regression lines of figure 1 show that the best growth in Trial 1 after 18 days of treatments was supported by the control algal diet (100 % on shell length basis) with the other diets following in the order: 85 % *C. calcitrans* + 15 % microcapsules (93 %) > 65 % *C. calcitrans* + 36 % microcapsules (85 %) > 50 % *C. calcitrans* + 50 % microcapsules (77 %).

In Trial 3, larvae fed microcapsules did relatively worse than starved larvae when the initial larval size was 105.70 µm, but they showed a greater growth than starved larvae when the initial sizes were 163.30 µm and 237.76 µm.

TABLE VI

The relationship between dry weight/organic weight and shell length of *C. gigas* larvae in trial 1

Diet	*Day*	*Dry wt. ngxlarvae^{-1}*	*Ash-free dry wt. ngxlarvae^{-1}*	*% ash-free dry wt. of dry wt.*	*Shell length (µm)*
	Initial	74.895	26.745	35.70	80.00
C. calcitrans	18	9038.500	3343.000	36.98	312.32
85 % *C. calc* + 15 % Microc.	18	13014.000	4375.500	33.62	289.38
65 % *C. calc* + 35 % Microc.	18	8630.000	2855.500	33.08	265.53

Performance of larvae fed the control algal diet and the artificial diet were not significantly different when the initial larval size was 163.30 µm ($p < 0.001$) (Table V).

The relationship between ash-free dry weights and sizes of larvae fed different diets in Trial 1 is shown in Table VI. Larvae fed the mixed diets were heavier for size than when fed *C. calcitrans* only, but had a slightly lower % ash-free dry weight of dry weight content.

The lipid, carbohydrate and protein content of larvae in Trial 1, initially and at the end of the experiment (day 18) for the control algal diet and the algal diet substituted with 15 % and 35 % microcapsules (by weight), are presented in figure 2 as a percentage of ash-free dry weight (organic weight). Table VII shows the dry weight, ash-free dry weight and biochemical composition on a per larva basis.

Survival rates in Trial 1 ar shown in figure 3.

Larval numbers and mortalities in Trials 1,2 and 3 are shown in Tables VIII, IX and X respectively.

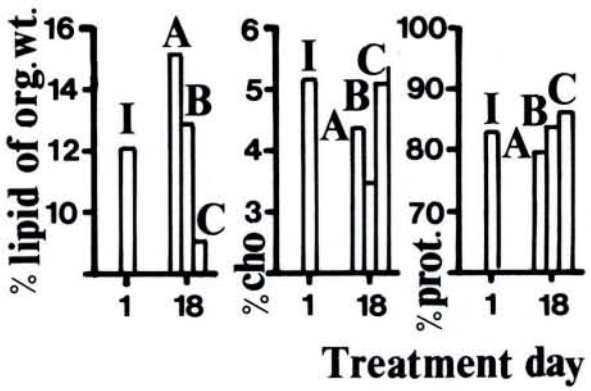

FIG. 2. – Biochemical composition of *C. gigas* larvae fed on 3 different diets in trial 1. (I: Initial; A: *C. calcitrans*; B: 85 % *C. calc.* + 15 % Microc.; C: 65 % *C. calc.* + 35 % Microc.) (cho: carbohydrate).

TABLE VII

Dry weight and biochemical composition of larvae on a per larva basis. Trial 1

Diet	*Day*	*Dry wt. ng×larvae^{-1}*	*Ash-free dry wt. ng×larvae^{-1}*	*Lipid ng×larvae^{-1}*	*CHO ng×larvae^{-1}*	*Protein ng×larvae^{-1}*
	Initial	74.895	26.745	3.255	1.431	22.059
C. calcitrans	18	9038.500	3343.000	525.518	159.473	2658.009
85 % *C. calc* + 15 % Microc.	18	13014.000	4375.500	564.533	168.420	3642.547
65 % *C. calc* + 35 % Microc.	18	8630.000	2855.500	259.007	147.630	2448.363

TABLE VIII

Larval numbers x 10^3 for each food treatment in trial 1 at 8 time periods

Period (day)	C. calc	*85 % C. calc. + 15 % microc.*	*65 % C. calc. + 35 % microc.*	*50 % C. calc. + 50 % microc.*	*Microc.*	*Starved*
1	350	350	350	350	350	100
4	204	119	140	70	18	41.5
6	140	15	26	5	0	18
8	104	14	21	4	–	0
11	89	12	13	4	–	–
13	53	12	9	3	–	–
15	44	8	9	2	–	–
18	40	0	6	1	–	–

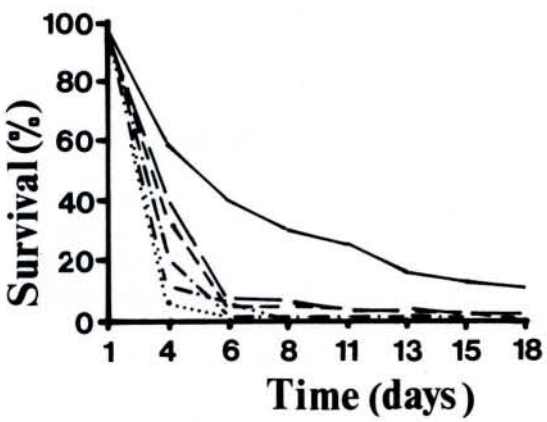

FIG. 3. – Survival rates of *C. gigas* larvae fed different treatments in trial 1. (—— *C. calc.*; ---- 85 % *C. calc* + 15 % Microc.; —— —— 65 % *C. calc.* + 35 % Microc.; — · — · 50 % *C. calc.* + 50 % Microc.; · · · · · Microcapsules; — · · — Starved).

TABLE IX

A) Larval numbers x 10^3 for each food treatment in trial 2 at 3 time periods

Period (day)	C. calc	*Microc. (7 mg · d^{-1})*	*Microc. (3.5 mg · d^{-1})*	*Microc. (14 mg · d^{-1})*	*Microc. (7x2 mg · d^{-1})*
1	10	10	10	10	10
4	2.6	2.1	2.3	2.3	2.9
6	2.1	0	0	0	0

B) % Larval mortality for each food treatment in trial 2 over 2 time periods

Period (day)	C. calc	*Microc. (7 mg · d^{-1})*	*Microc. (3.5 mg · d^{-1})*	*Microc. (14 mg · d^{-1})*	*Microc. (7x2 mg · d^{-1})*
1-4	74	79	77	77	71
4-6	19.2	100	100	100	100
MEAN	46.6	100	100	100	100

TABLE X

Larval mortality in trial 3 with different treatments

Initial size (µm)	*% larval mortality at the end of the experiment*		
	Algal diet	*Microc. diet*	*Starved*
105.70	4.11	38.91	4.95
163.30	68.98	82.16	83.07
237.76	16.50	78.58	24.44

DISCUSSION

Growth

In these trials, the microcapsules as a single diet did not support growth in the oyster larvae, and when they were fed mixed diets and the control algal diet, the growth at the end of the experiments was significantly different between all treatments. However, as the larvae grew their ability to utilise the microcapsules increased, and when they reached a mean shell length of 163.30 µm the artificial diet supported a growth rate similar to that of the control algal diet.

The growth as a percentage of the control diet was higher than that of the capsule supplementation. Substitution of the algal diet with 50 % microcapsules supported 77 % (on a shell length basis) of the control algal diet growth. This

could initially suggest the microcapsules were being assimilated and that they may have some nutritional value. However, the fact that the growth rate decreased as the microcapsule substitution of the diet increased indicates that the artificial diet was nutritionally inadequate.

The lack of some nutritional factor, such as essential polyunsaturated fatty acids could be an explanation. However, even 50 % of *C. calcitrans* in the diet should supply these (WEBB & CHU, 1981). Thus, it seems more likely that factors such as poor digestibility have to be considered.

Size of microcapsules could have influenced their better use by bigger larvae. It is possible that poor performance of smaller larvae was due to an adverse effect of the wide size range and presence of larger (20 µm) capsules.

Biochemistry

The increased percentage of lipid (as an ashfree dry weight) in relation to the initial values in those diets supporting better growth during the experimental period, and the marked drop in the percentage of lipid shown by diets supporting unsatisfactory growth, agrees with the results reported by HOLLAND & SPENCER (1973) and HAMILTON (1986). It suggest that the increased percentage of lipid is due to its storage as an energy source at metamorphosis, and that a decrease is related to a nutritional imbalance.

The percentage levels of carbohydrate and protein did not correlate with growth, which suggests that relative amounts of carbohydrate and protein are less important for maintaining good growth (WEBB & CHU, 1981).

Mortality

In general, mortality values were in proportion to the percentage of microcapsule supplementation in the diets, although mortalities were overall higher than expected even with the control algal diet. Therefore, lower mortality values may have been obtained under better conditions when feeding microcapsules. Those larvae that survived grew reasonably well.

Break-down of microcapsules and associated bacterial contamination could have had a role in poor performance of larvae fed the artificial diets. This is supported by the high settling rate shown by the microcapsules.

In some cases, higher mortalities were observed in larvae fed the single microcapsule diet than those for starved larvae. This could be due to an extra stress produced by filtration induced by the presence of the capsules.

In general, these results seem to indicate that the nutritional composition of the artificial diet tested here was not suitable for the oyster larvae, and that the nutritional requirements of oyster spat are different from those of oyster larvae, as the artificial diet supported better growth, similar to that produced by the control algal diet, when the larvae reached a certain size in Trial 3.

If these results are confirmed in further works, despite the fact that the use of artificial diets with oyster spat represents a potential saving in commercial hatchery rearing, microencapsulated diets would have to be developed and designed to be more acceptable and more nutritionally valuable not only to oyster spat, but also to larvae in order to represent a potential saving for both the running and investment costs involved in mass culture of algae.

A more detailed and precise knowledge of the nutritional requirements of oysters is also likely to be achieved when an artificial diet with a known nutritional composition can be succesfully used.

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SUMMARY

The present work was carried out to assess the nutritional value of a new type of microcapsule produced by FRIPPAK FEEDS, as a single diet and mixed in different proportions with the algal species used as the control diet, *Chaetoceros calcitrans* (Paulsen) Takano, for larvae of *Crassostrea gigas*.

The microcapsules as a single diet did not support growth in the oyster larvae. When the larvae were fed mixed diets and the control diet, the growth at the end of the experiments was significantly different between all treatments. As the larvae grew their ability to utilise the microcapsules increased, and growth of larvae fed the control algal diet and the artificial diet were not significantly different when the initial larval size was 163.30 µm.

Substitution of the algal diet with 50 % microcapsules supported 77 % (on a shell length basis) of the control algal diet growth.

The differences in food value have been explained in terms of growth and biochemical composition of the larvae. Gross biochemical composition of the microcapsules and the algal diet was also considered.

RESUMEN

ESTUDIO DEL VALOR NUTRITIVO DE UN NUEVO TIPO DE DIETA MICROENCAPSULADA PARA LARVAS DE OSTRA DEL PACÍFICO (*Crassostrea gigas*, THUNBERG). El presente trabajo se llevó a cabo para evaluar el valor nutritivo de un nuevo tipo de microcápsulas producidas por FRIPPAK FEEDS, específicamente para larvas de moluscos bivalvos, como dieta única y mezclada en diferentes proporciones con la especie de alga utilizada como dieta control, *Chaetoceros calcitrans* (Paulsen) Takano, para larvas de *Crassostrea gigas*.

Las microcápsulas, como dieta única, no dieron lugar a crecimiento alguno en las larvas de ostra. Cuando éstas fueron alimentadas con las dietas mixtas y la dieta control, el crecimiento al final del experimento fue significativamente diferente entre todos los tratamientos. Confome las larvas crecían, se incrementó su capacidad para utilizar las microcápsulas, y cuando el tama-

ño inicial de las larvas fue de 163,30 µm, el crecimiento de aquellas alimentadas con la dieta control no fue significativamente diferente al de las alimentadas con la dieta artificial.

La suplementación de la dieta algal con 50 % de microcápsulas produjo un 77 % del crecimiento de la dieta control (según la longitud de la concha).

Las diferencias en el valor nutritivo se explican en términos de crecimiento y composición bioquímica de las larvas. También se considera la composición bioquímica de las microcápsulas y de la dieta algal.

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