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Current Status of Microencapsulated Diets for Aquaculture

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ABSTRACT

The replacement of live foods by artificial diets in larval culture remains one of the major problems of commercial aquaculture because many systems are still dependent upon one or even two live food-chain links to provide adequate nutrition for larval molluscs, crustaceans, and fish. Recently it has been demonstrated that one, and in some cases, both live food-chain links may be successfully replaced by microencapsulated diets.

The technology, which has reached pilot-scale production in at least one instance, now includes a wide range of coacervation and interfacial polymerization methods producing individual capsules ranging from 2 μm in diameter to capsule aggregates. These have been designed to function either as complete nutrient delivery systems or as feed supplements. In addition, by incorporation of specific nutrients into microcapsules, it is now possible to determine essential nutritional requirements for a wide range of aquatic larvae.

The present paper reviews the progress in these fields and considers their likely consequences for aquaculture.

Index Entries: Microencapsulated diets, for aquaculture; diets, microencapsulated for aquaculture; aquaculture, microencapsulated diets for.

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INTRODUCTION

Since the initial application of microencapsulation techniques to solve marine larval feeding problems, research in this field has grown to the extent that there are now groups working in the United States, Europe and Japan employing a variety of methods (Table 1). The technology now includes a wide range of coacervation and interfacial polymerization techniques producing individual microcapsules ranging from 2 μm in diameter to capsule aggregates, designed to act either as complete nutrient systems or as feed supplements.

Many commercial aquaculture systems are still dependent upon culturing one or even two live food-chain links to supply adequate nutrition for larval molluscs, crustaceans, and fish. The aim, to replace these live foods with artificial diets, has met with limited success in the total replacement of live foods (Table 2). However, it has recently been demonstrated that at least one live stage can be successfully replaced with

TABLE 1
Microencapsulation Methods Used for Marine Larval Feeds

	Method	Source	Modified by
1.	*Interfacial polymerization*		
	Diaminohexane–sebacoyl chloride	Chang et al. (1966)	Jones et al. (1974) Jones (1978) Clark et al. (1982)
	Diaminohexane–dichlorodiethyl ether	Suzuki et al. (1968)	Clark et al. (1982)
	Protein–terephthaloyl chloride	Kondo et al. (1976)	Clark et al. (1982)
2.	*Coacervation*		
	Gelatin–acacia	Green and Schleicher (1957)[a]	Gatesoupe and Luquet (1977a) Langdon and Waldock (1981) Holland and Jones (1981)
	Ethyl cellulose	Vrancken and Claeys (1970)[a]	Langdon and Waldock (1981)
	Zein–alcohol	Brynko and Bakan (1963)[a]	Gatesoupe and Luquet (1977a)
3.	*Microglobules*		
	Zein-coated diets	Bayliss (1975)[a] Gatesoupe and Luquet (1977a)	Clark et al. (1982)

[a]Patents

microencapsulated diets (*1*). The present paper reviews the progress in this field and considers the required development of microencapsulated diets for aquaculture.

MATERIALS AND METHODS

The microcapsules used in assimilation and gustatory trials with larval guppies (*Poecilia reticulata*) (Tables 3, 4) and in some feeding trials with the post larval goby (*Pomatoschistus minutus*) were of the nylon crosslinked protein-wall type described by Chang et al. (*2*). Their preparation and modification is described in Sakamoto et al. (*1*).

Gelatin–acacia capsules were used for some gustatory trials with larval guppies (Table 4) and for feeding experiments with guppies and gobies (Figs. 2 and 3). These were prepared using a modification of the method by Green and Schleicher (US Patent 2,800,457) described in full in Langdon and Waldock (*3*).

Encapsulated diets used in the assimilation and gustatory experiments consisted of homogenized newly hatched *Artemia* (San Francisco strain) in a 4:1 ratio with chicken egg. Diets used in guppy and goby feeding experiments are described in Sakamoto et al. (*1*), with cod and pollack oils substituted for *Tapes* oil.

Microcapsules used for feeding directly to fish were 40–100 μm diameter and fed at a concentration of 15–17/mL to groups of 8–10 fish larvae in 1.5 L beakers containing aerated filtered fresh (guppy) or seawater (goby). Microcapsules for feeding *Artemia* were 10–30 μm diameter and fed at a concentration of 3–500/mL to 24 h-old *Artemia* nauplii. These were harvested by filtration through 100 μm nylon mesh after 24 h, and fed at a concentration of 15–17/mL to larval fish.

The efficiency of assimilation of ingested microcapsules and live food was estimated using the ratio method of Conover (*4*). Percentage daily growth rate of larval guppies was calculated using the method of Winberg (*5*).

The total lipid fraction of the diets and whole bodies of *Artemia* was extracted by the method of Folch et al. (*6*), using 2:1 v/v chloroform:methanol containing 0.01% w/v of the antioxidant 2,6-di-*tert*-butyl-*p*-cresol. Total lipids were separated into neutral and polar lipid fractions by silicic acid column chromatography (*7*), and fatty acid methyl esters prepared using boron trifluoride in methanol (*8*). Fatty acid methyl esters were separated by gas–liquid chromatography on a Carlo-Erba 4160 gas chromatograph equipped with a 25-m glass capillary column of 0.5 mm id coated with SP 1000. Fatty acids were identified using commercially available standards (C.S. Chromatography Services, Merseyside, UK) and graphical techniques.

TABLE 2
Commercial Fish and Shellfish Species Cultured on Microencapsulated Diets

Species	Capsule Type	Results	Author
Mollusca	Nylon-protein, glycopeptide	Fed as protein–carbohydrate–cholesterol diet to spat, promoted growth	Gabbott et al. (1975)
Crassostrea gigas	Gelatin–acacia	Fed as lipid supplement to spat, enhanced growth	Langdon and Waldock (1981)
Crustacea			
Macrobrachium rosenbergii	Nylon–protein	Survival to 4th stage on incomplete diet	Jones et al. (1975)
Penaeus merguiensis	Nylon–protein	Survival to mysis 1 on incomplete diet	Jones et al. (1975)

P. plebejus	Nylon–protein	First-stage zoea survival, expts. still in progress	Preston, personal commun.
P. monodon	Nylon–protein	5% Survival to post larva	Jones, unpub. data
P. japonicus	Nylon–protein	50% Survival to post larva	Jones et al. (1979)
Fish			
Scophthalmus maximus (Turbot)	Nylon–protein	Survival through early larval stages	Dye, personal commun. Jones, unpub. data
Pleuronectes platessa (Plaice)	Acid dichloride–protein	17.5% Survival after metamorphosis	Adron et al. (1977)
Dicentrarchus labrax (Sea Bass)	Zein-coated	4% Survival	Gatesoupe et al. (1977b)
Solea solea (Sole)	Zein-coated	7% Survival	Gatesoupe et al. (1977b)

TABLE 3
Assimilation and Growth Rates of Encapsulated Diets Fed to Larval and Juvenile Guppy (*Poecilia reticulata*)

Diet	Ash to[a] dry weight, %	Assimilation efficiency[b] by larval guppy, % mean and standard error	Assimilation efficiency[b] by juvenile guppy, %	Daily growth rate,[c] %	
				Larval	Juvenile
Artemia nauplii	3.28	91.8 ± 3.46	52.5	4.01	2.06
Encapsulated *Artemia* extract	1.5	90.27 ± 2.35	86.9	0.24	0.82
Fry food (commercial)	12.5	80.1 ± 5.12	82.2	3.54	2.61
Encapsulated fry food	2.24	80.1 ± 6.4	87.9	0.38	0.74

[a]Two replicates.
[b]Three replicates. Assimilation efficiency calculated from Conover (1966).
[c]Experiments with groups of 10 fish; daily growth rate calculated according to Winberg (1960).

TABLE 4
Summary of Trials with Gustatory Stimulants Added to Microcapsules and Fed to Larval Guppies

Percentage ratio of numbers of capsules eaten against total taken into the mouth:	
Live *Artemia* (control)	100%
Encapsulated *Artemia*	24%
Encapsulated + inosine	44%
Encapsulated + L-methionine	67%

RESULTS

Table 2 shows some results obtained by feeding microencapsulated diets direct to marine larvae. Although oyster larvae may benefit from lipid supplements fed in microcapsules, recent work (*9*) indicates that bacteria may be important in acting as a nutrient supply or aiding breakdown and digestion of the capsules.

Amongst the commercially important crustaceans, only *Penaeus japonicus* shows a high survival rate on encapsulated diets (*10*). Best survival rates for larval fish on encapsulated diets (17.5%) have been achieved for the plaice (*11*), although several other commercial species have reached the postlarval stage on capsules (*12*).

In an attempt to determine the cause for poor growth and survival on encapsulated diets, groups of larval guppies were fed the same foods in encapsulated and non-encapsulated form (Table 3). The efficiency of assimilation of the diets by larval and juvenile fish was measured together with the percentage daily growth on each diet. Results show that the process of encapsulation (nylon–protein) does not impair assimilation efficiency, and a higher percentage assimilation was achieved with larger capsules fed to juvenile guppies than with live *Artemia*. Despite similar assimilation efficiencies, growth rates were low for those groups of fish fed encapsulated diets.

The probable reason for poor growth is demonstrated by the feeding experiments shown in Fig. 1. In these experiments starved larval guppies were presented with either live *Artemia* or encapsulated diets containing the same food, and the number of strikes at food particles and subsequent ingestion recorded. It is clear that every *Artemia* attacked was ingested, and that after 10 min the attack rate declined markedly as fish became satiated. Although a similar attack rate was maintained upon microcapsules over the first 10 min, a large number of capsules were rejected, and the number swallowed by the larvae was much lower. Thus the continuing high attack rate over 30 min is a consequence of the low ingestion rate of capsules that fail to satiate the hunger of the fish. In

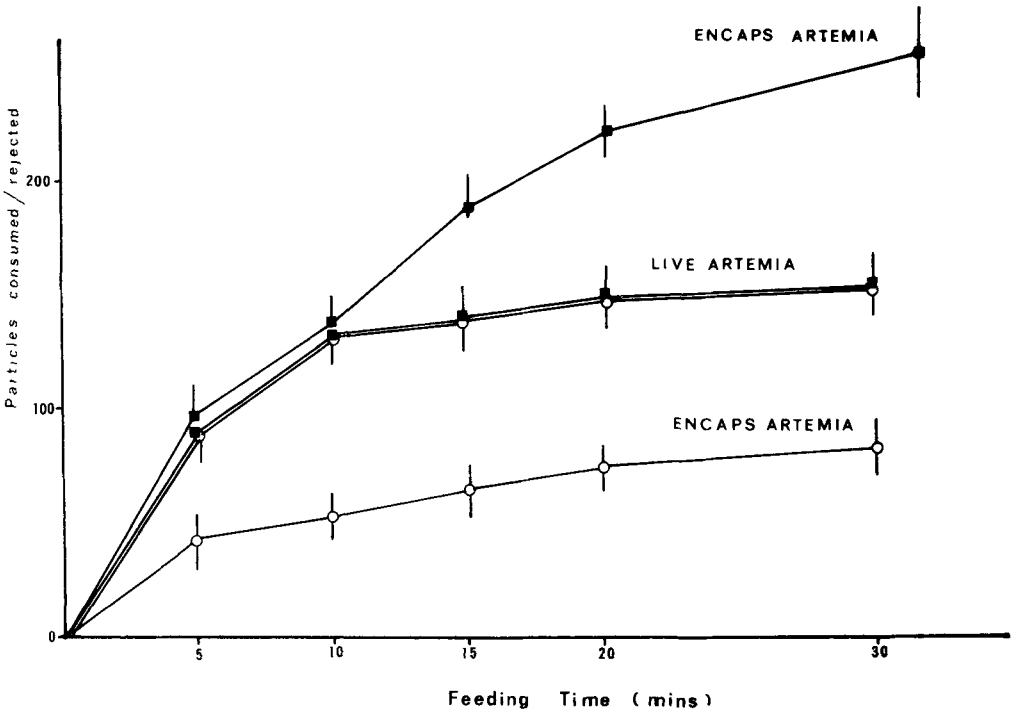

Fig. 1. Acceptance and rejection rates of live *Artemia* and encapsulated *Artemia* by larval guppies (*Poecilia reticulata*) (SD calculated on mean eight fish): ■, attack rates on diets; ○, ingestion rates of diets.

many cases larval fish were observed to take the capsule into the mouth, but to reject it almost immediately.

Fish are known to employ gustatory discrimination in feeding (*13*) and trials with gustatory stimulants (Table 4) have isolated the amino acids inosine and L-methionine, which significantly increase the ingestion rate of microcapsules for the guppy. These are, however, likely to vary for different fish and shellfish species. Similar tests with the goby revealed that only inosine and mussel extract invoked significantly higher ingestion rates.

Although the use of microcapsules as a direct larval food is limited to some extent by their lack of acceptability, their use indirectly to improve the nutritional value of conventional live foods such as *Artemia* and rotifers (*Brachionus*) is proving much more successful. It has been demonstrated that both of these live foods are often inadequate in promoting high growth and survival levels in marine larvae (*14*), and moreover may vary depending on their origin (*15*). In most cases nutritional inadequacy has been attributed to a lack of polyunsaturated fatty acids (PUFA), particularly 20:5ω3 and 22:6ω3, essential for the growth and survival of marine larvae (*16*). This is illustrated in Table 5, which shows the variation in fatty acid composition of total lipid in *Artemia* from different sources. Although total amounts of lipid do not vary greatly between the *Artemia*

strains, essential PUFA are at the trace level or absent in some races. To combat these deficiencies *Artemia* have been fed together with algae rich in PUFA (*17*), although this means setting up costly live algal cultures.

We have demonstrated that it is possible to modify the nutritional composition of *Artemia* over a short period (*1*). Figure 2 shows a further series of experiments in which post larval gobies (*Pomatoschistus minutus*) were fed on freshly hatched *Artemia* (San Francisco strain), and on *Artemia* prefed on simple diets containing cod and pollack oils. Groups fed on *Artemia* containing pollack oil maintained 100% survival and showed a significantly ($P < 0.05$) higher growth rate than groups fed on unfed *Artemia* or *Artemia* containing cod oil. Similar results were obtained with *Artemia* of the Florida strain.

Fatty acid analysis of the cod and pollack oil extracts (Table 6) reveals that although both oils contain high levels of 20:5ω3 and 22:6ω3, pollack oil is particularly rich in 22:6ω3.

TABLE 5
Total Fatty Acid Composition of Newly Released *Artemia nauplii* from Different Sources

Fatty acid	San Francisco	Spanish	Australian	Brazilian	Brazilian Macau
16:0	18.6	12.4	11.1	15.7	19.3
16:1ω7	7.9	20.1	10.4	12.9	8.7
16:2	1.9	—	—	—	0.7
16:3ω3	—	—	—	—	2.5
17:0	—	0.8	—	2.0	—
18:0	6.0	3.5	2.0	6.2	7.0
18:1ω9	32.0	16.9	14.9	23.5	28.9
18:1ω7	5.5	13.9	9.1	16.0	6.6
18:2ω6	6.1	5.1	8.2	3.3	8.0
18:3ω6	0.9	0.8	—	tr	5.4
18:3ω3	10.9	5.9	17.9	3.3	2.8
18:4ω3	1.9	1.3	7.8	tr	—
20:1ω9	—	0.9	0.4	tr	—
20:1ω7	0.8	tr	—	tr	0.6
20:2ω6	0.5	—	—	—	—
20:3ω6	—	0.2	—	tr	—
20:3ω3	0.5	—	tr	—	tr
20:4ω6	1.0	2.6	1.3	7.8	2.5
20:4ω3	0.6	0.2	0.8	—	—
20:5ω3	3.3	15.0	15.2	9.5	2.3
22:1	0.6	—	—	—	—
22:5ω6	0.4	—	—	—	tr
22:5ω3	—	—	—	—	1.1
22:6ω3	0.8	tr	0.7	—	3.6
Σ PUFA	26.9	31.1	51.9	23.9	28.2

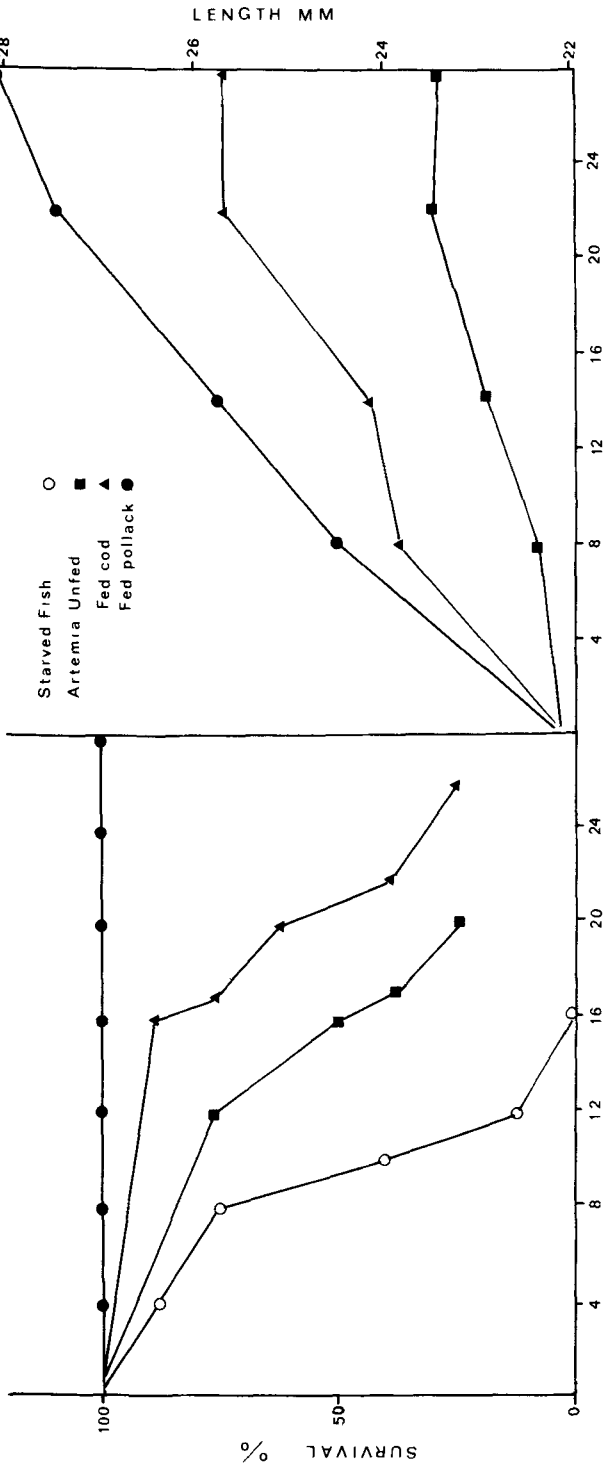

Fig. 2. Growth rates of post larval gobies fed on *Artemia*: ■, unfed; ▲, fed on cod oil diet; ●, fed on pollack oil diet.

Figure 3 shows the results of similar experiments conducted with fresh water guppy larvae. In this case the addition of PUFA fed to *Artemia* produces no significant improvement in growth rates over unfed *Artemia*. This is in agreement with Kanazawa et al. (*18*), who have demonstrated that several fresh water fish species have a significant capacity for bioconversion of 18:3ω3 to 20:5ω3 and 22:6ω3 and thus have less demand for these long chain highly unsaturated fatty acids in their diet.

DISCUSSION AND CONCLUSIONS

Figure 4 is an attempt to summarize the present status of microencapsulated diets in aquaculture. It is unlikely that they will play any direct role in bivalve culture, where algal foods may be supplied simply by pumping sea water containing 'wild' phytoplankton through culture vessels. However, Langdon and Waldock (*3*) have demonstrated that lipid supplementation by means of microcapsules is possible and have recently grown *Crassostrea virginica* on non-algal diet (*8*).

For commercial culture of marine fish and crustacean larvae, it is now possible to eliminate algal culture altogether and to enrich live foods such as *Artemia* and *Brachionus* with single feeds of encapsulated diets. These diets can be modified to include nutritional elements, such as PUFAs, that may be lacking in the live foods to ensure high survival and growth rates in commercial larval species. This is particularly important for aquaculture in developing countries where algal monocultures are often difficult to establish.

Already at least one company is producing a commercial dry free-flowing encapsulated food that is designed to act as a nutritional supplement to any strain of *Artemia*. Since most warm temperate and tropical countries possess their own *Artemia* strains (usually nutritionally inadequate), the application of such a diet will greatly reduce the cost of *Artemia* cysts because current sources are restricted at present. For fresh water invertebrate and fish culture, it seems unlikely that there will be such a high demand for encapsulated diets to supplement live foods since these are more likely to be nutritionally adequate. However, nutritional research in this field is inconclusive and nutritional requirements of individual species still remain to be elucidated.

Direct feeding of encapsulated diets, with the elimination of all live foods, still remains only partially successful. Though the process of encapsulation reduces bacterial contamination (*10*) and does not hinder assimilation, acceptability remains a problem. Most larvae strike at, and take microcapsules of the right size into the mouth, but rejection rates at this stage are still high. This is perhaps not surprising since studies have shown that the selection of specific food items is often critical for survival of fish larvae in the wild (*19*); thus it is to be expected that for many species food ingestion will only be triggered by very specific gustatory

TABLE 6
Composition of Fatty Acids in Pollack Oil and Cod Oil

Fatty acid	Pollack oil	Cod oil
16:0	20.140	23.150
16:1	7.720	9.484
18:0	6.535	3.333
18:1	25.720	31.890
18:2ω6	1.183	0.727
18:3ω3	0.855	0.500
18:4ω3	1.522	0.500
20:1	2.941	2.587
20:5ω3	11.770	12.050
22:1	1.896	—
22:5ω3	1.573	—
22:6ω3	9.288	6.500

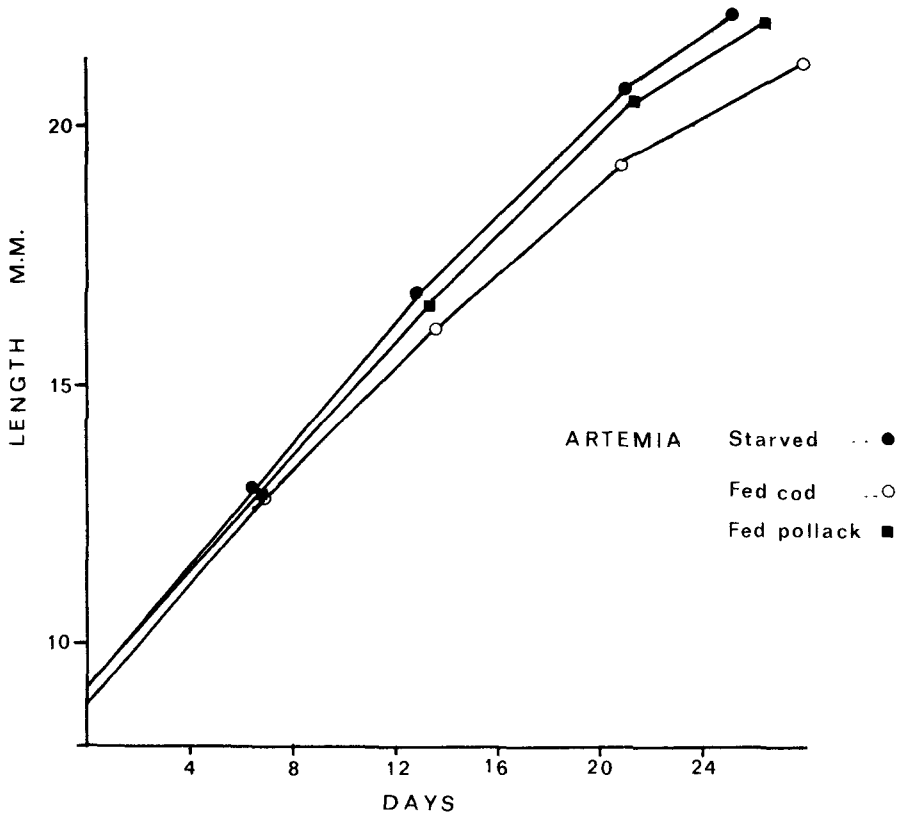

Fig. 3. Survival and growth rates of larval guppies fed on *Artemia* supplied with different diets: ●, fish fed on unfed *Artemia*; ○, fish fed on *Artemia* fed on cod oil diet; ■, fish fed on *Artemia* fed on pollack oil diet.

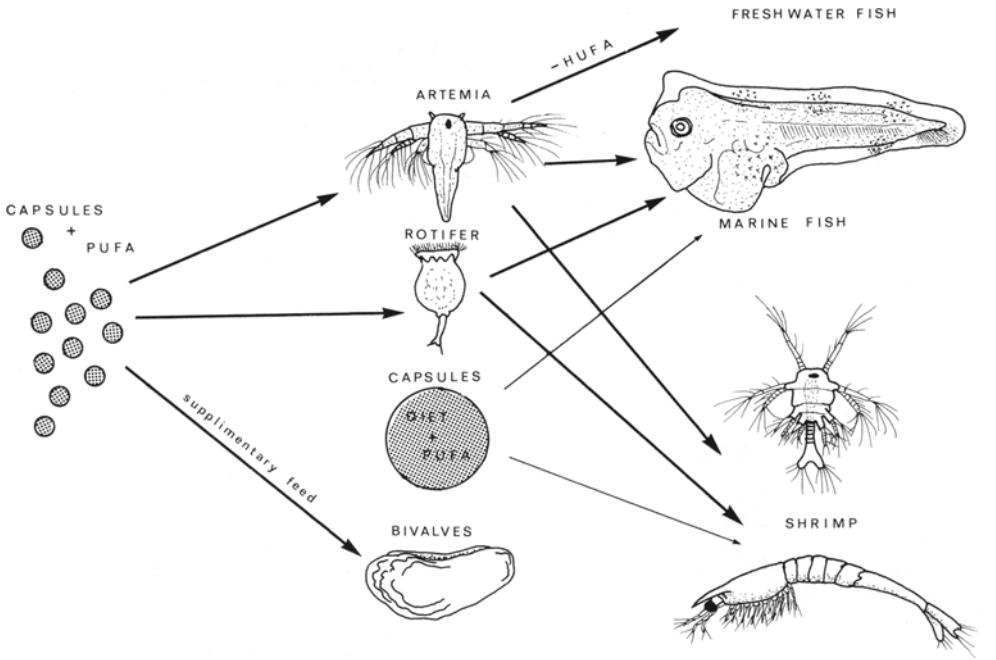

Fig. 4. Present status of microencapsulated diets in aquaculture.

stimuli. The success achieved with *Penaeus japonicus* larvae on microencapsulated diets results from its unselective mode of filter-feeding, which allows it to accept a wide range of food items (*20*). Progress in direct feeding of encapsulated diets will be dependent upon further detailed studies of gustatory stimuli invoking ingestion, particularly in predatory marine larvae.

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REFERENCES

1. Sakamoto, M., Holland, D. L., and Jones, D. A. (1982), *Aquaculture,* **28,** 311.
2. Chang, T. M. S., MacIntosh, F. C., and Mason, S. C. (1966), *Can J. Physiol. Pharmacol.* **44,** 115.
3. Langdon, C. J., and Waldock, M. J. (1981), *J. Mar. Biol. Assn. UK* **61,** 431.
4. Conover, R. J. (1966), *Limnol Oceanogr.* **11,** 338.
5. Winberg, G. G. (1960), *Fish. Res. Board Can. Transl. Series* No. **194,** Beloruss State Univ. Minsk.
6. Folch, J., Lees, M., and Sloane-Stanley, G. H. (1957), *J. Biol. Chem.* **226,** 497.
7. Christie, W. W. (1973), *Lipid Analysis, Isolation, Separation, Identification and Structure Analysis of Lipids.* Pergamon Press, Oxford, pp. 338.

8. Morrison, W. R., and Smith, L. H. (1964), *J. Lipid Res.* **5,** 600.
9. Langdon, C. J., and Siegfried, C. A. (1982) in *Symposium on Recent Innovations in the Cultivation of Pacific Molluscs.* San Diego, in press.
10. Jones, D. A., Kanazawa, A., and Rahman, S. A. (1979), *Aquaculture* **17,** 33.
11. Adron, J. M., Blair, A., and Cowey, C. B. (1977), *Actes de Colloques du C.N.E.X.O.* **4,** 67.
12. Gatesoupe, F. J., Girin, M., and Luquet, P. (1977), *Actes de Colloques du C.N.E.X.O.* **4,** 59.
13. Bardach, J. E., and Atema, J. (1971), in *Handbook of Sensory Physiology* Vol. 4, Beidler, L., ed., Springer-Verlag, New York, pp. 293–336.
14. Howell, B. R. (1979), *Aquaculture* **18,** 215.
15. Watanabe, T., Oowa, F., Kitajima, C., and Fujita, S. (1978), *Bull. Jpn. Soc. Sci. Fish.* **44,** 1115.
16. Holland, D. L. (1978), in *Biochemical and Biophysical Perspectives in Marine Biology,* Vol. 4, Malins, D. C., and Sargent, J. R., eds., Academic Press, London, pp. 85–119.
17. Scott, A. P., and Middleton, C. (1979), *Aquaculture* **18,** 227.
18. Kanazawa, A., Teshima, S., and Ono, K. (1979), *Comp. Biochem. Physiol.* **63B,** 295.
19. Hempel, G. (1975), in *The Early Life History of Fish,* Blaxter, J.H.S., ed., Springer-Verlag, New York, pp. 755–759.
20. Hirata, H., Mori, Y., and Watanabe, M. (1975), *Mar. Biol.* **29,** 9.