

Nutrition of Penaeid Prawns and Shrimps

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Abstract Since Hudinaga succeeded in the artificial hatching and subsequent culture of larvae of the prawn, *Penaeus japonicus*, techniques for rearing this prawn from hatching to commercial sizes have been improved in Japan and applied to other penaeid species in Asian and other countries. The nutritional requirements of *P. japonicus* juveniles started to be investigated about 15 years ago. As a result, this prawn is found to require proteins, lipids, carbohydrates, minerals, and vitamins for normal growth, indicating the deficiency disease, poor growth, and high mortality when reared with diets lacking some nutrients. On the basis of this knowledge, compounded artificial diets are used practically for commercial production of *P. japonicus* as substitutes for traditional live food such as the short-necked clam and mussel.

However, seed production of penaeids has depended on live food such as diatoms, *Chlorella* and *Artemia*. Mass culture of planktonic organisms not only requires much manual help and expensive equipment but also fluctuates with climatic conditions. Also, the nutritive value of planktonic organisms is occasionally variable and this makes the use of live food for mass culture restrictive. Therefore, the development of artificial diets for larval penaeids is one of the most important research areas in the field of penaeid culture. We have prepared microparticulate diets for larval penaeids for use both as substitutes for live food and for nutritional studies. In this presentation, I intend to deal with the overview of penaeid nutrition.

Introduction

Techniques for the artificial culture of crustaceans such as the prawn, *Penaeus japonicus*, the shrimp, *Macrobrachium rosenbergii*, and lobster, *Homarus americanus*, from hatching to commercial size have been established. However, some problems related to artificial diets and disease still remain. The nutritional requirements of *P. japonicus* have been manifested by the introduction of refined test diets by Kanazawa et al. (1970) and Deshimaru and Kuroki (1974a), and the prawn has been shown to necessitate adequate levels of proteins, lipids, carbohydrates, minerals and vitamins as do other aquatic animals. However, nutritional studies on other prawns and shrimps are few or fragmentary (Forster, 1976; New, 1976, 1980; Wickins, 1976; Hanson et al., 1977a, b; Ceccaldi, 1978; Kanazawa, 1980, 1982; Castell, 1982; Teshima, 1984).

This paper presents an overview of the nutritional requirements of penaeid prawns and shrimps.

Protein and amino acid requirements

Proteins are indispensable nutrients for growth and maintenance of life of all animals. Deshimaru and Yone (1978c) have pointed out that the prawn, *P. japonicus*, requires 52-57% protein for optimum growth and food efficiency. Kanazawa et al. (1981) have demonstrated that the shrimp, *Metapenaeus monoceros*, gave best growth with a diet containing 55% casein. Several groups of workers have reported the optimum protein levels in diets for *Penaeus indicus* (43%: Colvin, 1976), *Penaeus monodon* (46%: Lee, 1971; 40%: Aquacop, 1977; 40%: Khannapa, 1977; 35%: Bages and Sloane, 1981), *Penaeus aztecus* (23-31%: Shewbart et al., 1973; 40%: Venkataramiah et al., 1975), *Penaeus seti-*

ferus (28-32%: Andrews et al., 1972), *Penaeus californiensis* (31%: Colvin and Brand, 1977), *Penaeus vannamei* (30%: Colvin and Brand, 1977), *Penaeus stylirostris* (35%: Colvin and Brand, 1977), and *Penaeus merguensis* (50%: Aquacop, 1978; 34-42%: Sedgwick, 1979). Thus, the optimum protein levels in diets for prawns and shrimps are different among species. I assume that the diversity of optimum protein levels for crustaceans is likely to come from a variety of factors, namely, the discrepancy in food habits, ages of specimens, and protein sources used. In fact, Sick and Andrews (1973) have shown that soybean proteins are good protein sources for *Penaeus duorarum*. The content of essential amino acids (EAA) and balance of amino acids could be related to the nutritive value of proteins used (Kitabayashi et al., 1971b; Deshimaru and Shigueno, 1972; Deshimaru, 1982; Hew and Cuzon, 1982).

Kanazawa and Teshima (1981) have clarified by tracer techniques using radioactive acetate that *P. japonicus* requires 10 amino acids, i.e., arginine, methionine, valine, threonine, isoleucine, leucine, lysine, histidine, phenylalanine, and tryptophan, all of which are also EAA for various fish. The essential amino acids have also been demonstrated for other penaeids such as *P. monodon* (Coloso and Cruz, 1980) and *P. aztecus* (Shewbart et al., 1972). On the other hand, Deshimaru and Kuroki (1974c, 1975a, b) and Deshimaru (1982) showed that diets containing only amino acids instead of protein brought about a very poor growth and high mortality in feeding trials of *P. japonicus*.

Recently, the effects of dietary protein, lipid, and carbohydrate levels on the growth and survival of larvae of *P. japonicus* were examined by feeding trials using purified diet with carrageenan as a binder (Teshima and Kanazawa, 1984). As a result, the effects of protein levels on growth and sur-

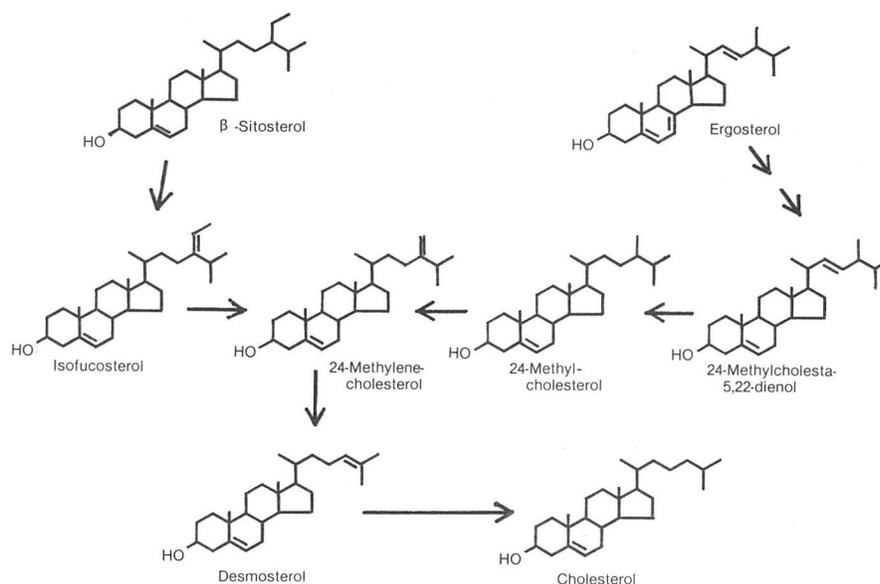


Fig. 1. Proposed mechanism for C-24 alkylation of C₂₈ and C₂₉ sterols in crustaceans. C₂₇ sterols: cholesterol and desmosterol; C₂₈ sterols: ergosterol, 24-methylcholesta-5, 22-dienol, 24-methylcholesterol, and 24-methylenecholesterol; C₂₉ sterols: β-sitosterol and isofucosterol.

vival of *P. japonicus* larvae varied with dietary carbohydrate levels but not with dietary lipid levels. The optimum protein levels for prawn larvae were estimated to be around 45%, 45-55%, and 55% or more when the diets contained 25%, 15%, and 5% levels of carbohydrate, respectively.

Cholesterol and other sterol requirements

Crustaceans require essential fatty acids (EFA) as also found for many fish species. However, the unique aspect of lipid nutrition in crustaceans is that they require dietary sources of sterol for normal growth and survival because of the absence of *de novo* sterol-synthesizing ability from acetate and mevalonate (Teshima and Kanazawa, 1971; Teshima, 1982). Feeding experiments using artificial diets

Table 1. Growth and survival of *Penaeus japonicus* larvae on purified diets with 0.5% level of each sterol.

Dietary sterol	Feeding period (day)	Survival rate (%)	Number of larvae			
			M ₁	M ₂	M ₃	P ₁
Sterol-free	9	30		29	1	
Cholesterol	9	74				74
7-cholestenol	9	37			30	7
22-dehydrocholesterol	9	60		19	41	
24-methylenecholesterol	9		19		41	
Ergosterol	9	68			6	56
24-methylcholesta-5,22-dienol	9	53			37	16
β-sitosterol	9	55		27	28	
Stigmasterol	9	11		3	8	
Fucosterol	9	2	2			
Isofucosterol	9	61			37	24
Lanosterol	9	2	2			

have shown that *P. japonicus* requires sterols for growth and survival, indicating an optimum level of 0.5% in diets (Kanazawa et al., 1971a; Shudo et al., 1971). Kanazawa et al. (1971b) have also demonstrated that *P. japonicus* could utilize to some extent ergosterol, β-sitosterol, and stigmasterol as a substitute for cholesterol. We presume that sterols other than cholesterol are utilized by *P. japonicus* after being converted to cholesterol in their bodies as proposed by Teshima and Kanazawa (1973) and Kanazawa et al. (1976a).

Recently, we succeeded in rearing larval *P. japonicus* using refined diets (Jones et al., 1979a; Kanazawa et al., 1982; Teshima et al., 1982a) with best growth and survival using a diet containing 1.0% cholesterol (Teshima et al., 1982b). Furthermore, we demonstrated that the dietary value of sterols other than cholesterol is inferior to cholesterol, but 24-methylenecholesterol, 24-methylcholesta-5, 22-dienol, and isofucosterol had a high dietary value (Teshima et al., 1983) (Table 1). Based on the dietary value of various sterols examined, we suspect that the dealkylation of C₂₈- and C₂₉-sterols to cholesterol (C₂₇-sterol) proceeds via the following pathways: β-sitosterol → isofucosterol → 24-methylenecholesterol; ergosterol → 24-methylcholesta-5, 22-dienol → 24-methylcholesta-5-enol → 24-methylenecholesterol; 24-methylenecholesterol → desmosterol → cholesterol (Fig. 1).

Essential fatty acid requirements

We have also shown the absence of *de novo* synthesis of linoleic (18:2ω6), linolenic (18:3ω3), icosapentaenoic (20:5ω3), and docosahexaenoic (22:6ω3) acids from acetate-¹⁴C or palmitic acid-¹⁴C in *P. japonicus* (Kanazawa and Teshima, 1977; Kanazawa et al., 1979b), in *P. monodon* (Kanazawa et al., 1979c), and in *P. merguensis* (Kanazawa et al., 1979c) (Table 2). These data suggest that crustaceans

may require some of these fatty acids as essential nutrients. In fact, Kanazawa et al. (1977b, 1978, 1979d, 1979f) have shown by feeding experiments that juveniles of *P. japonicus* gave a higher weight gain with diets containing 18:2 ω 6, 18:3 ω 3, 20:5 ω 3, or 22:6 ω 3 than 18:1 ω 9, indicating the necessity of ω 3- fatty acids, especially ω 3-highly unsaturated fatty acids (HUFA). The optimum levels of 20:5 ω 3 and 22:6 ω 3 for *P. japonicus* juveniles were found to be about 1.0% in diets (Kanazawa et al., 1979a) (Fig. 2). Shewbart and Mies (1973) also revealed that growth of *P. aztecus* was improved by the addition of 18:3 ω 3 to the refined diet, and that optimum growth was attained with diets containing 1% 18:3 ω 3. Fenucci et al. (1981) found a quadratic correlation between the rate of growth and the percentage of 18:3 ω 3 in the diet of juvenile *P. stylirostris*. Jones et al. (1979b) and Teshima and Kanazawa (1984) pointed out the necessity of ω 3- HUFA for growth and survival of the larval stages of *P. japonicus*.

Nutritive value of various lipids

Studies on EFA requirements for crustaceans have suggested that the nutritive value of lipids for prawns and shrimps is probably related to the types and content of EFA. High nutritive values of lipids rich in ω 3-HUFA, such as pollack liver oil and shrimp head oil, have been demonstrated for *P. duorarum* (Sick and Andrews, 1973). Kanazawa et al. (1977a) have pointed out that superior dietary value was obtained with marine lipids containing ω 3-HUFA such as pollack liver oil and short-necked clam oil, indicating that the inferior dietary value of soybean oil containing 18:3 ω 3 is possibly due to the shortage of ω 3-HUFA such as 20:5 ω 3 and 22:6 ω 3. Guary et al. (1976a) also showed a high nutritive value of sardine oil and short-necked clam oil for *P. japonicus*. Aquacop (1978) reported that cod liver oil sustained growth and survival of *P. merguensis* as the best source of lipid.

On the other hand, Deshimaru et al. (1979) have shown that a good lipid source for *P. japonicus* diets was a mixture of soybean oil-pollack liver oil (6% in diets; 1:3 or 1:1, w/w). Also, Colvin (1976b) has reported that a mixture of wheat germ oil and peanut oil was best for *P. indicus* among the vegetable oils examined.

As stated above, the types and content of EFA dominate the nutritive value of dietary lipids. However, other lipid components such as phospholipids (see section 5) and sterols should be considered in evaluating the dietary value of lipids for prawns and shrimps.

Effects of dietary phospholipids on growth and survival

Since short-necked clam oil had better growth-promoting effect for *P. japonicus* than pollack liver oil, we intended to clarify the compounds responsible for such an effect. Several lipid fractions were isolated from short-necked clam oil, and the growth-promoting effect was examined by adding 1% of each lipid fraction to a diet containing 7% pollack liver oil as lipid source. As a result, lecithin fraction had the highest ef-

Table 2. Proportional radioactivity in individual fatty acids constituting polar lipids isolated from *Penaeus monodon* and *Penaeus merguensis* 24 hr after injection of acetate-1-¹⁴C.

Fatty acid	% Distribution of radioactivity*	
	<i>P. monodon</i>	<i>P. merguensis</i>
14:0	0.2	2.4
15:0	0.8	0.1
16:0	13.6	50.0
17:0	1.4	1.2
18:0	11.2	18.5
20:0	0.3	2.0
16:1	9.1	6.1
17:1	1.8	0.1
18:1 ω 9	37.3	10.2
20:1 ω 9	9.7	2.6
18:2 ω 6	0.1	0.3
20:2 ω 6	1.2	5.9
20:3 ω 6	1.1	0
20:4 ω 6	7.8	0.1
20:3 ω 3		
22:5 ω 6	0.4	0
18:3 ω 3	0	0
20:4 ω 3	0.2	0
20:5 ω 3	0.5	0.1
22:5 ω 3	0.7	0.1
22:6 ω 3	1.1	0.2

*Fatty acids from polar lipid fractions were subjected to argentation TLC as methylesters, and then the methylesters of saturated, monoene, diene, triene, tetraene, pentaene, and hexaene fatty acids were subjected to preparative GLC on 10% DEGS followed by radioactive measurements of trapped samples.

fect, followed by the cephaline fraction (Kanazawa et al., 1979e).

Recently, Teshima et al. (1982b), by using microparticulate diets containing carrageenan as a binder, noticed that the inclusion of phospholipids in diets is indispensable to growth and survival of larval *P. japonicus*. When maintained on diets without supplemental lecithin, the larvae suffered 100% mortality before reaching the mysis stage. Hence, Kanazawa (1982, 1983) further examined the effects of various phospholipids on growth and survival of larval *P. japonicus*. Growth and survival of the prawn larvae were found to be improved by the addition of 1% soybean lecithin, bonito egg lecithin, and soybean phosphatide linositol, whereas no beneficial effect on growth and survival was demonstrated with 1% dipalmitoylphosphatidylcholine, phosphatidylethanolamine (from bovine brain and bonito egg), phosphatidylserine (from bovine brain), sphingomyelin (from bovine brain), cytidine-5'-diphosphate choline, or taurocholic acid. These results suggest that the requisite for effective phospholipids is to possess choline or the inositol group besides unsaturated fatty acids as fatty acid moieties.

Little is known why dietary sources of phospholipids are effective in enhancing or sustaining growth and survival of larval and juvenile *P. japonicus*. I assume that dietary phospholipids may be required due to a specific requirement for some phospholipids for both the smooth transport of dietary lipids, particularly cholesterol, in the hemolymph and a slow rate of phospholipid biosynthesis.

Nutritive value of carbohydrates

The addition of large amounts (more than 10%) of glucose to diets generally reduces growth of prawns such as *P. aztecus* (Andrews et al., 1972), *P. duorarum* (Sick and Andrews, 1973), and *P. japonicus* (Deshimaru and Yone, 1978b; Abdel-Rahman et al., 1979). Abdel-Rahman et al. (1979) have shown that *P. japonicus* juveniles had a better weight gain on diets containing disaccharides such as sucrose, maltose and trehalose, and polysaccharides such as dextrin and starch, than on diets containing monosaccharides such as glucose, galactose and fructose. They thought that the reason why dietary di- and polysaccharides had a higher nutritive value than monosaccharides for *P. japonicus* is that dietary glucose is quickly absorbed from the stomach and released all at once into the hemolymph. Therefore, when large amounts of glucose were added to diets, blood glucose levels were elevated to abnormally high levels. Disaccharides and polysaccharides are not absorbed from the stomach, but are digested to glucose and trehalose in the midgut and hepatopancreas which are then released gradually into the hemolymph. Dietary disaccharides such as maltose are thus effectively utilized as an energy source. Aquacop (1978) suggested that a carbohydrate such as starch appears more suitable than glucose. Pascual et al. (1983) have also demonstrated that the addition of sucrose or dextrin as a carbohydrate source for *P. monodon* juveniles was better than other carbohydrates as shown in Table 3.

Table 3. Mean survival rate and weight gain of *Penaeus monodon* juveniles fed various carbohydrate-containing diets after 6 weeks of rearing. Numbers in parentheses represent initial stock.

Carbohydrate	% Survival		Ave. % weight gain			
	Level (%)	10	40	10	40	
Dextrin	(23)	36	(23)	23	24	34
Maltose	(23)	35	(23)	0	5	—
Sucrose	(23)	56	(22)	38	7	28
Molasses	(22)	0	(21)	0	—	—
Sago palm starch	(22)	42	(22)	0	7	—
Cornstarch	(23)	27	(22)	0	7	—
Cassava starch	(23)	0	(21)	0	—	—

There are conflicting results on the effect of supplemental glucosamine on growth and survival of *P. japonicus*. Kitabayashi et al. (1971a) have demonstrated that addition of 0.52% glucosamine to diets improved growth but that of chitin inhibited growth. On the other hand, Deshimaru and Kuroki (1974b) have pointed out that a dietary source of glucosamine is unnecessary for *P. japonicus* juveniles and it inhibits the growth-promoting effect of cholesterol. Thus, the role of dietary glucosamine is still not clear.

Mineral requirements

Prawns and shrimps may absorb some minerals from the water to some extent, but they may necessitate a dietary source of some minerals for growth because of repeated loss of certain minerals during molting. Deshimaru et al. (1978)

and Deshimaru and Yone (1978a) have shown that *P. japonicus* takes up calcium from sea water and does not require calcium, magnesium and iron. Kanazawa et al. (1984) have revealed that addition of calcium to diets could be necessary to maintain the ratio of calcium-phosphorus (1:1) in diets, although growth of *P. japonicus* on diets with and without calcium supplement is comparable. Kitabayashi et al. (1971a) have also pointed out the importance of the Ca/P ratio, indicating an optimum ratio of 1:1 for *P. japonicus*. Huner and Colvin (1977) have shown Ca/P ratios of 2.2:1 to be optimum for growth of juvenile shrimp, *P. californiensis*. Shewbart et al. (1973) considered that calcium, potassium, and sodium chloride are not necessary for *P. aztecus*, but phosphorus may be essential. The necessity of phosphorus has been manifested with *P. japonicus* (Kitabayashi et al., 1971a; Deshimaru et al., 1978a; Kanazawa et al., 1984). Deshimaru et al. (1978a), have reported that *P. japonicus* requires phosphorus (2.0%), potassium (1.0%), and trace metals (0.2%). Kanazawa et al. (1984) have shown that this species requires calcium (1.0%), phosphorus (1.0%), magnesium (0.3%), potassium (0.9%), and copper (0.6%) in dry diets. As mentioned above, there is some conflict on the published values for the requirement of prawns for calcium and magnesium. Since it is likely that the effect of calcium varies according to types of calcium salts used such as primary, secondary, and tertiary salts, the calcium requirement of prawns should be reevaluated by a more detailed experiment. The addition of iron (0.006%) and manganese (0.003%) inhibited growth of *P. japonicus* juveniles.

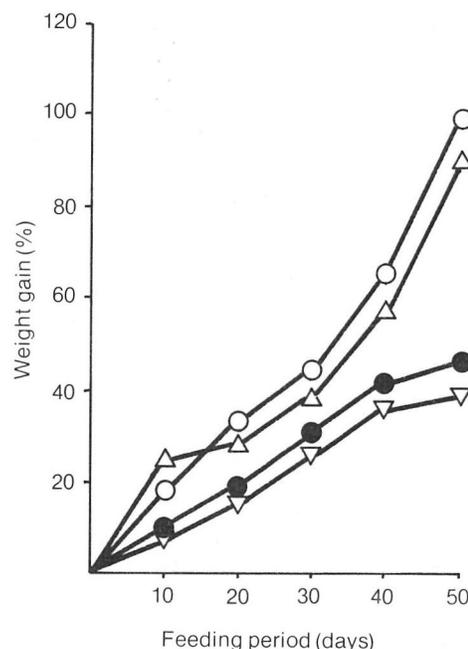


Fig. 2. Dietary requirements of *Penaeus japonicus* juveniles for icosapentaenoic acid.

- ▽ 5.0% 18:1ω9
- 4.5% 18:1ω9 + 0.5% 20:5ω3
- 4.0% 18:1ω9 + 1.0% 20:5ω3
- △ 3.0% 18:1ω9 + 2.0% 20:5ω3

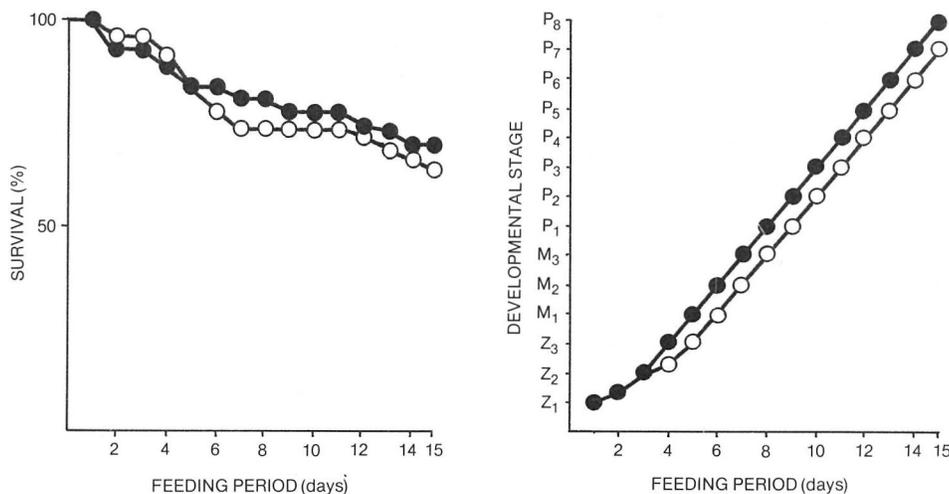


Fig. 3. Mass production of *Penaeus japonicus* larvae with microparticulate diet.
 ○*Chaetoceros* + *Artemia* + commercial diet; ● microparticulate diet.

Vitamin requirements

Several workers (Kanazawa et al., 1976b; Guary et al., 1976b; Deshimaru and Kuroki, 1976, 1979) have shown that *P. japonicus* juveniles require about 300-1,000 mg of ascorbic acid, 60 mg of choline, 200-400 mg of inositol, 6-12 mg of thiamine, and 12 mg of pyridoxine, per 100 g of diet, respectively. Lightner et al. (1977) have found that *P. californiensis* and *P. stylirostris* sometimes show an abnormal symptom, named "black death," with a characteristic blackening of the esophagus wall, cuticle, gastric wall, hind gut wall, and gills. "Black death" has been recognized as a symptom of ascorbic acid deficiency (Magarelli et al., 1979) with a dietary intake of 0.1% sufficient to prevent nutrition-related deaths among these shrimp (Lightner et al., 1979). It has been suggested that juvenile *P. californiensis* require dietary ascorbic acid to form adequate amounts of collagen from the unhydroxylated precursor, procollagen (Hunter et al., 1979). Also, depletion/repletion of ascorbic acid in whole body tissue was studied in *P. californiensis* and *P. stylirostris* (Magarelli and Colvin, 1978). On the other hand, Sedgwick (1980) has reported the requirements of *P. merguensis* for vitamin and mineral supplements in diets based on freeze-dried *Mytilus edulis* meal.

Recently, Kanazawa et al. (unpublished data) also examined the requirements of larval *P. japonicus* for various vitamins by using microparticulate diets with carrageenan as a binder. As a result, the prawn larvae were found to require vitamin E, nicotinic acid, choline, pyridoxine, biotin, folic acid, ascorbic acid, cyanocobalamin, vitamin D, inositol, riboflavin, thiamine, and β-carotene. The shortage of one of these vitamins resulted in the cessation or retardation of metamorphosis and in high mortality during larval development. Further studies have been done on the quantitative requirements of larval *P. japonicus* for several vitamins. The requirements for some vitamins such as ascorbic acid were apparently higher for *P. japonicus* larvae than for juveniles. It is conceivable, however, that some vitamins may have leached into the water before feeding. This means that the

vitamin requirements of larval *P. japonicus* mentioned above should be regarded as "practical demand for rearing of the larvae."

Seed production with microparticulate diets

As mentioned above, the nutritional requirements of prawn larvae are studied by using microparticulate test diets. Recently, microparticulate diets were used as substitutes for live foods such as diatom and *Artemia* in seed production of *P. japonicus* (Villegas and Kanazawa, 1980; Kanazawa and Teshima, 1983; Kanazawa, 1985). From zoea 1 stage, the larval prawn reached postlarva 8 using only microparticulate diets. As a result, 21,000 postlarvae (survival rate of 70%) were produced in a 16-ton tank (Fig. 3).

Conclusion

The nutritional requirements of *P. japonicus* have been well investigated using purified or semi-purified diets. In this species, the requirements for proteins, lipids, carbohydrates, vitamins, and minerals have been manifested, and the accumulated knowledge has been useful in the commercial production of prawn diets. On the other hand, there is little information on the nutritional requirements of other prawns and shrimps. Further nutritional studies should be conducted on commercially important species to make formula feeds with a high dietary value. Another important subject is the manifestation of nutritional requirements in the larval stages of prawns and shrimps to achieve their successful mass production with artificial diets.

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