Crustacean nutrition and larval feed, with emphasis on Japanese spiny lobster, Panulirus japonicus

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Crustacean nutrition and larval feed, with emphasis on Japanese spiny lobster, *Panulirus japonicus*

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Abstract Crustacean nutritional studies, such as requirements for proteins, amino acids, lipids, fatty acids, vitamins, minerals, etc., are reviewed, concerning with the fact that the feeding requirement of crustaceans differs between species (for example, carnivorous species require feeds with high contents of protein and lipid, compared with omnivorous and herbivorous species). This information is useful in determining the optimum dietary level of some nutrients. However, little information is available on the nutritional requirements of the spiny lobster, *Panulirus japonicus*, in particular during the phyllosoma stage.

So far, it has been established that only *Artemia* and gonads of the blue mussel, *Mytilus galloprovincialis*, are effective as foods for phyllosoma. It is very difficult to rear phyllosoma to reach to the puerulus stage with a high survival rate, even though phyllosoma are fed these kinds of feeds. It shows a lack of information on some nutritional elements in *Artemia* and mussel. The aims of our studies are to search for or develop optimal foods for the phyllosomal stage of spiny lobster *P. japonicus*. Studies on making an artificial phyllosomal feed were started with checking the size, softness, leaching, etc. of the artificial feed by rearing phyllosoma. Seasonal variations in proximate compositions of gonad of blue mussel were also monitored.

This paper provides useful information in preparing the artificial feed for phyllosoma stage of spiny lobster.

Key words: crustacean, larval feed, nutrition, spiny lobster, *Panulirus japonicus*

Crustacean nutrition

This session reviews the nutritional requirements of crustaceans and their characteristics such as requirements for protein, amino acids, lipids, fatty acids, vitamins, minerals, *etc*., referring to some recent reports. It has been known that the feeding requirements of crustaceans differ between species (for example, carnivorous species require feeds with high contents of protein and lipid, compared with omnivorous and herbivorous species). This information is useful in determining the optimum dietary level of some nutrients. However, little information is available on the nutritional requirement of spiny lobster *P. japonicus*, in particular about the phyllosoma stage.

Protein requirement

Table 1 shows the published protein requirements of penaeid shrimps. To date, the protein requirements of many species have been investigated. Optimal protein levels of diets vary from 23-57%. Protein requirements of shrimps present quite large variations from one species to another. Protein requirements of *Metapenaeus macleayi* appear to be the lowest (27%), *Litopenaeus setiferus* following with 28-32%. Kuruma shrimp, *Marsupenaeus japonicus*, one of the important commercial shrimps cultured in Japan, requires 40-57% of protein in the diet. Protein level can be

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as high as 55% in commercial kuruma shrimp feed. However recently we found that protein level can be reduced from 55% to 45 or 50% by the addition of probiotics in diet or in rearing water (Mochizuki and Takeuchi, unpublished data). The nutrient digestibility in shrimp, and the environmental bacterial stability as well as water conditions might be improved. Protein requirement of black tiger, *Penaeus monodon*, the most popular aquaculture species, is lower than that of kuruma shrimp. White shrimp, *Litopenaeus vannamei*, requires about 40% protein in diets. Such inter-specific variability can in part be explained by the different feeding habits of different species of shrimp, and it suggests that the protein requirements need to be studied for specific species.

Crustaceans require ten kind of essential amino acids, such as arginine, methionine, valine, threonine, isoleucine, leucine, lysine, histidine, phenylalanine, and thryptophan. These characteristics are the same as that of fish.

Carbohydrate requirement

Kuruma shrimp requires 24% maltose in diet (Kanazawa, 1995). In general, shrimps require disaccharides (such as maltose, trehalose, *etc.*) and polysaccharides (such as dextrin, starch, glycogen, *etc.*), rather than monosaccharides (such as glucose, galactose, *etc.*). In particular, more than 20% glucose supplementation may retard the growth of kuruma shrimp. Beneficial effects of di- and poly-saccharides on crustaceans are the same as that on fish, such as carp and red sea bream. It is well known that the absorption speeds of glucose derived from saccharides relates to the turnover rate of carbohydrate metabolism such as glycolysis relates to the gluconeogenesis in fish and shellfish.

Lipid requirement

Table 2 shows the published requirements for essential fatty acids (EFA) and their biosynthesis in crustaceans. Kuruma shrimp requires 6.5-16.5% lipids in the diet. EFA requirement of kuruma

Table 1. Published requirements of protein for penaeid shrimp

Species	Protein requirement (% dry matter)	Reference
Metapenaeus macleayi	27	Maguire and Hume, 1982
M. monoceros	55	Kanazawa *et al.*, 1981
Marsupenaeus japonicus	40-57	Balazs *et al.*, 1973
		Deshimaru and Kuroki, 1975
		Deshimaru and Yone, 1978
		Teshima and Kanazawa, 1984
Penaeus monodon	35-50	Lee, 1971
		Bages and Sloane, 1981
		Alava and Lim, 1983
		Bautista, 1986
		Shiau *et al.*, 1991
Farfantepenaeus aztecus	40-51	Venkataramiah *et al.*, 1975
		Zein-Eldin and Corliss, 1976
F. californiensis	35	Colvin and Brand, 1977
Litopenaeus setiferus	28-32	Andrews *et al.*, 1972
L. merguiensis	50-55	Aquacop, 1978
L. vannamei	38-40	Smith *et al.*, 1985
		Pedrazzoli *et al.*, 1998
L. indicus	43	Colvin, 1976
L. esculentus	40	Hewitt, 1992
L. plebejus	47	Aquacop and Cuzon, 1992
L. stylirostris	35	Colvin and Brand, 1977

(Allan and Smith, 1988; partially modified)

Table 2. Published requirements and biosynthesis of essential fatty acids for crustaceans

Species	EFA requirement and Biosynthesis	Reference
Post-settlement		
Marsupenaeus japonicus	1% (DHA=EPA>LNA>LA)	Kanazawa *et al*., 1979
Penaeus chinensis	(DHA>AA>LNA>LA)	Xu *et al*., 1994
P. monodon	1% DHA (LNA⇏ EPA⇋ DHA)	Merican and Smith, 1996
Farfantepenaeus aztecus	1% LNA	Shewbart and Mies, 1973
Macrobrachium borellii	LNA⇏ EPA; 20:3n-6⇏ AA	Gonzalez-Baro *et al*., 1998
Panulirus japonicus	EPA⇏ DHA	Kanazawa and Koshio, 1994
Larvae		
Scylla serrata	0.8% n-3HUFA (rotifer feeding) 0.7-0.9% EPA and 0.5-0.7% DHA LNA⇏ EPA; LA⇏ AA (*Artemia* feeding)	Suprayudi *et al*., 2002 Suprayudi *et al*., 2004
S. paramamosain	1.3-2.5% EPA and 0.5% DHA (*Artemia* feeding)	Kobayashi *et al*., 2000
Portunus tribuberculatus	0.9-1.7% HUFA (rotifer feeding)	Takeuchi *et al*., 1999
Phyllosoma		
Panulirus cygnus	Long-chain PUFA (*Artemia* feeding)	Liddy *et al*., 2005
Jasus edwardsii	Need high percentage of DHA (*Artemia* feeding)	Nelson *et al*., 2004
Broodstock		
Macrobrachium rosenbergii	1.3% LA and 1.5% n-3 HUFA	Cavalli *et al*., 1999

shrimp juvenile is 1% docosahexaenoic acid (DHA) or eicosapentaenoic acid (EPA), but a higher requirement is needed for linolenic acid (LNA). *P. monodon* also requires 1% DHA. On the other hand, *Farfantepenaeus aztecus* requires 1% LNA. As for fatty acid metabolism, larval kuruma shrimp were found to have a greater ability than juvenile to convert LNA into n-3 highly unsaturated fatty acids (n-3HUFA), such as EPA and DHA. In other shrimps, *Penaeus chinensis* cannot convert LNA into EPA and EPA into DHA, while *P. monodon* can convert EPA into DHA. Larval crabs, such as the mangrove crabs, *Scylla serrata* and *S. paramamosain*, require n-3HUFA. They need higher levels of n-3HUFA at *Artemia* feeding stages than that at rotifer feeding stages, and also they cannot convert LNA into EPA and linoleic acid (LA) into arachidonic acid (AA). Examined via radioactive tracer experiments, Kanazawa and Koshio (1994) pointed out that spiny lobsters may lack the capacity for the bioconversion of EPA and DHA into other fatty acids. So, it can be considered that DHA and/or EPA are essential fatty acids for spiny lobster. In particular, phyllosoma of *Panulirus cygnus* and *Jasus edwarsii* need high levels of n-3HUFA or DHA during the *Artemia* feeding stages.

Phospholipids are also essential for crustaceans. Phosphatidylcholine (PC) and phosphatidylinositol (PI) are required, while phosphatidylethanolamine (PE) and phosphatidylserine (PS) have no nutritional effect. Most crustaceans require 0.5-1% of phospholipids in diet, except for freshwater prawn, *Macrobrachium rosenbergii* (Coutteau *et al*., 1997).

Cholesterol requirement in crustaceans is about 1% in larvae and 0.5% in juveniles. Shrimps are deficient in biosynthetic capability of sterol. But C_{28} and C_{29}-cholesterol can be converted into C_{27}-cholesterol *in vivo* (Kanazawa and Teshima, 1971; Teshima, 1998).

β-Carotene is an important nutrient for crustaceans. The usual pigments isolated from crustaceans are astaxanthin, β-carotene, echinenone and canthaxanthin. β-Carotene is converted into astaxanthin via cryptoxanthin, echinenone or canthaxanthin, respectively (Linan-Cabello and Paniagua-Michel, 1998). On the other hand, the pathway of bioconversion of the main dietary carotenoids in shrimps and *Artemia* is producing retinoic acid. It is well known that retinoic acid is one of the elements incurring abnormality. Overdose of vitamin A and retinoic acid causes bone deformity in fish. However, it has never been investigated in studies on lobsters. β-carotene is also a precursor of vitamin A. Addition of vitamin A in feeds could improve the growth of larval shrimps and be essential for normal development of the ovaries. The recommended supplementation level of vitamin A in shrimp feed is about 1500 IU/100 g dry weight of diet (Linan-Cabello *et al*., 2002), that is nearly the same as fish.

Vitamin and mineral requirements

Nutritional requirements of vitamins and minerals are similar to fish (Kanazawa, 1995; Shiau, 1998; Pedrazzoli *et al*., 1998). Recommended supplementation level of ascorbic acid (vitamin C) is 1% in the diet. This value is 100 times higher than that of dietary requirement. It is mainly on account of the behavior of shrimp feeding. Before shrimp ingest the feed, nutritional elements, especially water soluble vitamins, are leached. Therefore a high level supplementation of vitamin C is required. More interesting results of the studies on vitamin C requirement of crustaceans showed that kuruma shrimp and spiny lobster lack biosynthetic capacity of vitamin C, while American lobster has. Nearly the same results are obtained in studies on fish. Rainbow trout do not have the ability of biosynthesis of vitamin C, while carp have. Thus, it is necessary to check the biosynthetic capacity of vitamin C in each species of crustacean, and to examine the supplementation levels of the water soluble nutrients in the diet.

Calcium (Ca) and phosphorus (P) ratio is also important for crustaceans. Kuruma shrimp and American lobster require 1:1 and 1:2 of Ca:P, respectively (Kanazawa, 1995). It is well known that most crustaceans and fish can absorb Ca directly from seawater.

To date, the nutritional studies on crustaceans have focused on the investigation of shrimps. Little information has been reported concerning about nutritional requirements of lobsters, especially about phyllosoma and juveniles of spiny lobster *P. japonicus*. Further research is needed to clarify the nutritional requirement of phyllosoma.

Seed production and quality of foods in spiny lobster *Panulirus japonicus*

Seed production of spiny lobster has been conducted and puerulus and first stage juvenile

Table 3. Puerulus and juvenile production in Minamiizu Station

Year	Phyllosoma 1.5mm Times of Molting	Phyllosoma Duration	Puerulus 3cm	Juvenile
1990	30 (29-31)	292.2 (280-304)	2	2
1991	28.4 (25～30)	286.4 (266-306)	5	4
1992	29.0 (20～31)	317.5 (231-417)	67	21
1993	27	293	1	0
1994	26.6 (26-27)	308.8 (234-393)	144	54
1995	-	280.7 (256-314)	6	2
1996	-	312.0 (237-385)	59	31
1997	-	318.2 (256-385)	41	30
1998	-	373.0 (253-517)	113	49
1999	-	360.5 (321-405)	14	5
2000	-	338.3 (281-408)	17	7
2001	-	338.7 (316-370)	3	1
2002	-	328.6 (240-426)	120	78
2003	-	360.3 (288-537)	51	39
In total	20-31	231-537	643	323

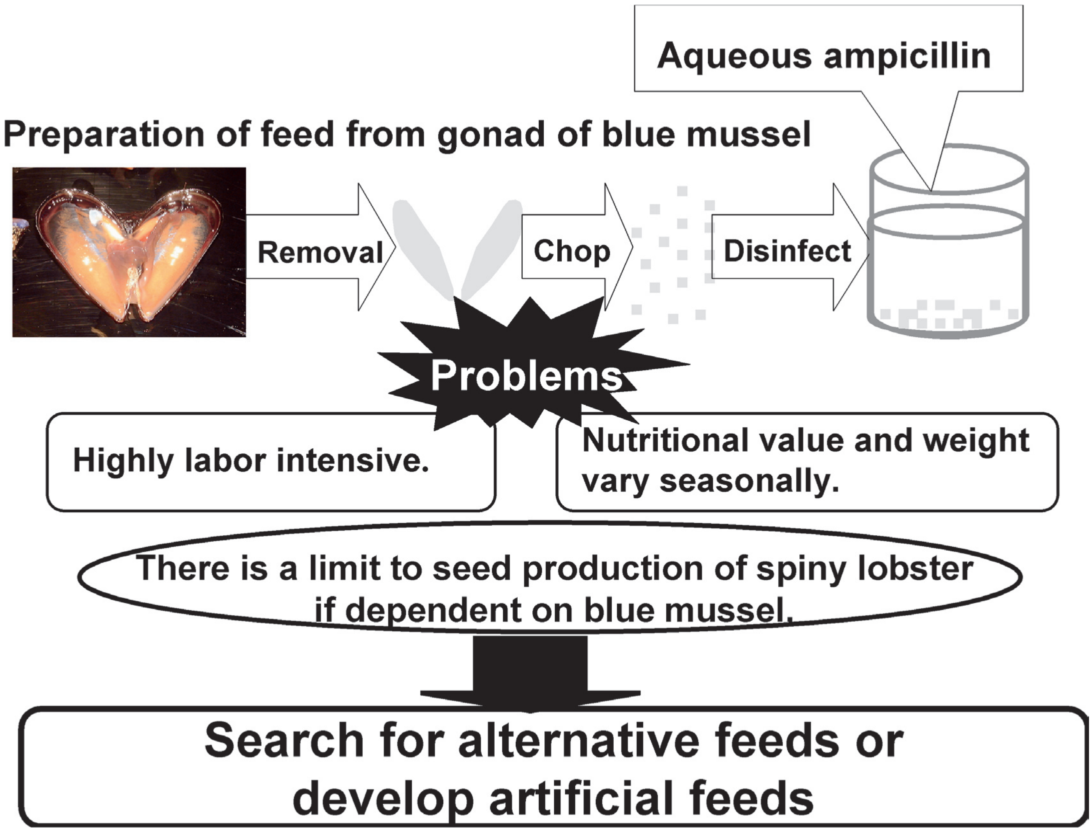

Fig. 1. Process of the preparation of feed for phyllosoma

have been obtained since 1990 (Table 3). Duration of phyllosoma is nearly 300 days, and molting times are about 30. Then phyllosoma metamorphose into puerulus. Only about 50% of puerulus can be successfully metamorphose to the juvenile stage. During the approximate 14-day puerulus stage, the larvae do not feed. So the quality of phyllosoma feed is very important for obtaining first lobster juvenile. So far, it has been known that only *Artemia* and gonads of blue mussel *Mytilus galloprovincialis* are effective as phyllosoma feeds. It is very difficult to make phyllosoma attain to puerulus at a high survival rate, even though phyllosoma were fed these kinds of feeds. It shows a lack of information on some nutritional elements in *Artemia* and mussel gonads. The aims of our studies are to search for or develop optimal foods for phyllosomal stage of spiny lobster *P. japonicus*. Studies in making an artificial phyllosomal feed were started with checking the size, softness, leaching, *etc.* of the artificial feed by reared phyllosoma. Seasonal variations in proximate compositions of gonads of blue mussel were also monitored.

Problems of blue mussel feeding

The preparation process for phyllosomal feed has met some problems (Fig. 1). The preparation process includes several steps: first, the shells of live blue mussels have to be opened; and then the gonads removed from the shells; after that the gonads are chopped into small pieces with a knife; finally in order to prevent infection, the minced gonads are immersed in 20 ppm ampicillin solution over night.

These processes is not only labor intensive, but also the nutritional values of the live mussel gonads of each lot needs to be checked because of their seasonal variation. Mass seed production depending on blue mussel gonads will be extremely difficult.

So it is necessary to search for alternative foods and to develop artificial feed. If phyllosoma could be fed on alternative foods, these foods will be easy to obtain in large quantities throughout the year and easier to prepare than blue mussel gonad. If phyllosoma could be fed on artificial feed, it will be easy to adjust the dietary size to changes in the phyllosomal size, easy to regulate the nutrient composition, and save labor.

Rearing conditions are also important for successful rearing of phyllosoma. So far, it is very difficult to make mass production of spiny lobster, because phyllosoma are very fragile. For example, when several individual phyllosoma are reared together in a small vessel, the long legs of individual phyllosoma interweaved each other and easy to be broken, and survival of phyllosoma is easy to be influenced by deterioration of the water condition due to uneaten food, etc. Recently, a new type of vessel - rotary vessel, was developed. This rotary vessel was shown to be effective to decrease the intertwine of individuals and increase the chance of meeting with feeds. In seed production, survival rate of larvae from phyllosoma to juvenile increased from 10% to 30% at the maximum. Also it is very important to introduce the hygienic treatment of rearing water, such as ultraviolet radiation, disinfectant, *etc*. Now rearing methods are being improved.

Seasonal variation of blue mussel

In seed production, phyllosoma are fed with *Artemia* enriched with *Phaeodactylum* during phyllosomal stages I to V. Then, phyllosoma are fed with gonad of blue mussel together with *Artemia*, but mainly with gonad of blue mussel. After the spawning season, the volume of mussel gonad becomes low. Since ovary is more effective compared with testis, usually testis and small size gonad are not used as phyllosoma feed.

Year-round monitoring and comparison of nutritional compositions were conducted. Harvested blue mussels were divided into two groups (samples 1 and 2, S1 and S2) by visual check. Usually, only S1 were used in seed-production. Difference in ratio of ovary weight/shell length was found between S1 and S2 (Fig. 2). In all seasons, values of S1 are higher than that of S2. The ratio decreased during the spawning period, especially in S1. Fig. 2 also shows the seasonal variation of crude ash, glycogen

Table 4. Nutritional levels of blue mussel Mytilus edulis during a year-round

Protein	30-70%	(Min.: Apr.)
Glycogen	0.5-40%	(Min.: Jan.)
N-3HUFA	1.5-3.9 g/100g	(Min.: Apr.)
Amino acids		(Min.: Dec.)

Table 5. Proposal for the development of artificial feeds for phyllosoma during the period of spiny lobster seed production project

Year	Research details
2005-2006	Analyzing effective compositions of blue mussel, alternative and natural foods
2005-2007	Gaining a clear understanding of feed shape for chewing and screen for alternative feeds
2006-2008	Developing new artificial feeds to achieve the process of metamorphosis and molting

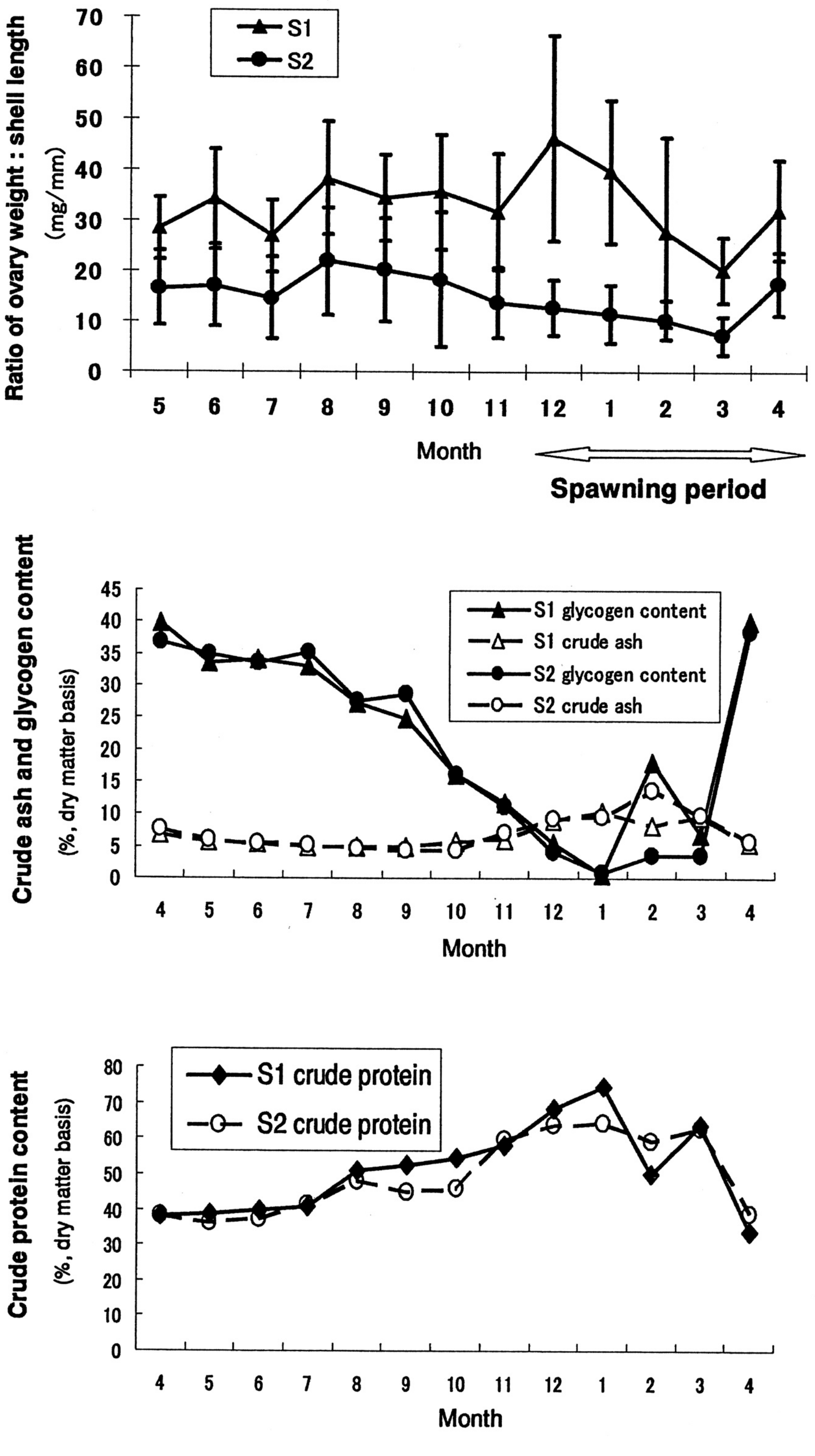

Fig. 2. Seasonal variation in ratio of ovary weight / shell crude (in upper figure), ash and glycogen content (in middle figure), and crude protein content (in lower figure)

content, and crude protein. Glycogen content in both S1 and S2 groups reached the maximum of 40% in April whereas the minimum of 0.5% in January. Crude protein content in both S1 and S2 groups reached the maximum of 70% in January whereas the minimum of 30% in April. The seasonal variation between crude protein and glycogen contents showed the contrary results. N-3HUFA in total lipids in both S1 and S2 groups reached the maximum of 3.9 g/100 g in January whereas the minimum of 1.5 g/100 g in April (Table 4). These patterns are the same as crude protein content (Fig. 2). Polar lipid contents in gonad mussel also showed the same pattern. On the other hand, amino acid contents reached the minimum in the spawning period. This result is the same as glycogen content. So all the nutritional contents have seasonal variations and showed wide ranges. We recommend that the best season for blue mussels to feed to phyllosoma is autumn, just before spawning, considering the nutritional balance of each element. The each value of blue mussel in this season is as follows; ratio of ovary weight/shell length, 30-35 mg/mm; crude protein content, 55-60%; glycogen content, 10-15%; polar lipids, 4.5-5.0%; nonpolar lipids, 7-8%; and n-3HUFA, 3.0 g/100 g.

Conclusion

From this year, we start a project of semi-mass production of spiny lobster. The subject is the development of artificial feeds for phyllosoma (Table 5). Nutritional compositions of blue mussel, alternative and natural foods are planned to be analyzed in 2005 and 2006. For example, seasonal variations of proximate compositions, amino acids compositions, lipid classes and fatty acid compositions of blue mussel have been investigated and the best season of high mussel quality has been found, as mentioned above. Some other kinds of natural foods will also be analyzed.

We aim to have a clear understanding of feed shape for chewing, and select ideal alternative feeds before the end of 2007. Our final goal is to develop a new artificial feed to achieve the process of metamorphoses and molting not later than 2008.

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