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NUTRITIONAL QUALITY OF LIVE FOOD ORGANISMS AND THEIR ENRICHMENT

ISSUED ON THE OCCASION OF THE WORKSHOP ON
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ORGANISED BY
THE CENTRE OF ADVANCED STUDIES IN MARICULTURE, CENTRAL MARINE FISHERIES RESEARCH INSTITUTE, COCHIN
Indian Council of Agricultural Research
HELD AT COCHIN ON 20TH AND 21ST DECEMBER 1985

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NUTRITIONAL QUALITY OF LIVE FOOD ORGANISMS AND THEIR ENRICHMENT

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PREFACE

Nutritional research on cultivable species of aquatic animals has been receiving greater attention all over the world. Live food organisms such as phytoplankton and zooplankton are successfully used for rearing the larvae of fish and shellfish in hatcheries. Using the knowledge of nutritional requirements of candidate species of cultured organisms, compounded feeds are being developed with locally available raw materials. Techniques have been sucessfully evolved to prepare microencapsulated diets for rearing larvae of fish and shellfish. Continued efforts are on to produce nutritionally well balanced feeds through deeper understanding of amino acid and fatty acid requirements of animals. As a major cost-factor in the production, feed is a very important component in the overall development of mariculture.

Prof. Takeshi Watanabe, Laboratory of Fish Nutrition, Tokyo University of Fisheries, Tokyo, Japan, who is an authority in fish nutrition, visited this Institute, as expert consultant on fish and shellfish nutrition at the Centre of Advanced Studies in Mariculture for a brief period during November-December 1985, to offer advice and suggestions and to upgrade research and education in fish and shellfish nutrition. During this period, scientists and post-graduate students had extensive group discussions on various topics. Dr. Watanabe organised and conducted a two day workshop on Nutritional Quality of Live-food organisms and their Enrichment. The course programme covered in detail, the nutritional quality of different live food organisms available for use in aquaculture. It highlighted the effect of culture techniques used for producing the live feeds on their nutritional quality. Food organisms cultured using certain culture media are deficient in highly unsaturated fatty acids (HUFA) which are very essential for the growth of fish and shellfish larvae. Feeding of such deficient food organisms to larvae may result in large scale mortality. Dr. Watanabe developed techniques to enrich these food organism with lipids rich in HUFA which can be incorporated into the food organisms.

This manual was prepared in connection with the workshop conducted by Dr. Watanabe. The nutritional quality of different live-food organisms were discussed and the techniques of enrichment of these food organisms with lipids are described in detail in this publication. It is hoped that this manual will be immensely beneficial to scientists, students and entrepreneurs involved in rearing of fish and shellfish larvae and hatchery production of their seed.

I express my sincere thanks to Prof. Takeshi Watanabe for preparing this valuable manual and for making various suggestions regarding fish and shellfish nutrition. I thank Shri Syed Ahamad Ali, scientist and counterpart to Prof. Watanabe for the assistance given in the preparation of the manual as well as for the keen interest shown in the conduct of the workshop.

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1

INTRODUCTION

Upto the present, more than 300 different species of finfish and shellfish have been cultivated throughout the world and the number of species is still increasing every year with the advancement of rearing techniques and methods for mass production of live feeds. Especially after finding the suitability of rotifers as the initial live food for hatched larvae in 1965, mass seedling techniques have been developed very rapidly. The use of rotifers was pioneered by Japanese workers some time ago. The most suitable diets for various developmental stages of some fish according to our present knowledge, are as follows: For freshly hatched fish of body length greater than 2.3 mm, rotifers are given as the initial diet and this is continued for about 30 days after hatching. When fish reach 7 mm or more in body length, marine copepods such as *Tigriopus, Acartia, Oithona* and *Paracalanus* or in their absence, *Moina* and *Daphnia* of fresh water origin, are fed to larvae together with rotifers, as rotifers are slightly smaller for larvae of 7 mm size. Brine shrimp *Artemia,* distributed commercially, are very frequently used as food for larvae of many marine fishes when there is shortage of marine copepods. Larvae larger than 10 to 11 mm are fed on minced fish, shellfish and shrimps or an artificial diet. When juveniles attain 30–50 mm in total body length, larval production is considered to be completed.

Thus, rotifers have been used most extensively and are very important as the initial live food for rearing larval fish. At present, without the mass culture of rotifers, larval rearing of marine fishes would be virtually impossible, although artificial microdiets are being gradually developed to replace costly live feeds. This important organism, systematically mass produced, has made fish larval production possible. However, some problems have been encountered regarding the dietary value of these live feeds. One of these problems concerns rotifers and the *Artemia.*

Rotifers were mass cultured on marine *Chlorella* as a feed organism until baker's yeast *Saccharomyces cerevisiae,* was also found to be very suitable for this purpose. When baker's yeast was used as food for rotifers, the density of the rotifer culture reached about 10 times higher than that was obtained by using marine *Chlorella.* Thus, recently, yeast has been used increasingly as food for rotifers as the production of juvenile fish has increased progressively in Japan from year to year.

However, the rotifers cultured with yeast frequently resulted in sudden heavy losses of larval fish (Kitajima and Koda, 1976; Fujita, 1979; Fukusho, 1977; Kitajima, 1978; Kitajima *et al.,* 1979). These high mortalities could be prevented by culturing the rotifers with both yeast and marine *Chlorella,* or by culturing the rotifers with yeast and then feeding them on marine *Chlorella* for a short period before feeding them to the fish (Kitajima and Koda, 1976; Fukusho, 1977; Kitajima *et al.,* 1979). This was one of the most important findings for the mass production of juvenile fish.

Our recent investigation on the nutritional quality of live feeds has demonstrated that the content of essential fatty acids (EFA) in the live feeds is the principle determinant of their dietary value. The purpose of this account is to review the nutritional quality of live feeds from the viewpoint of EFA for fish and shrimps and the methods of enrichment of live feeds for improving their dietary value. The nutrient contents such as minerals (Watanabe *et al.,* 1978 a) and essential aminoacids and protein quality (Watanabe *et al.,* 1978 b) of various live feeds were reviewed in Watanabe *et al.* (1982).

2

NUTRITIONAL QUALITY OF LIVE FOODS

Recent studies on EFA in fish have demonstrated that (Table 1) the EFA requirements of fish differ considerably from species to species. Rainbow trout require fatty acids of the linolenic family (ω3) as EFA (Castell *et al.*, 1972; Watanable *et al.*, 1974), whereas carp, eel and chum salmon require not only linolenic, but also linoleic acid for good growth (Watanable, 1982). On the other hand, these fatty acids did not meet the EFA requirement of marine fish, and highly unsaturated fatty acids, such as 20:5ω3 and 22:6ω3, (ω3HUFA) had to be supplied as EFA for them (Yone, 1978; Yone and Fujii, 1975), although these ω3HUFA were also found to be very effective for freshwater fish species except for *Tilapia* (Watanabe, 1982). Based upon these results recently obtained in various fish on EFA, the dietary value of live feeds was discussed in this section.

TABLE 1. *Essential fatty acid requirement of fish*

Species	Requirement	Reference
Rainbow trout	18:3 ω3 1%	Castell *et al.* (1972)
	18:3 ω3 0.8%	Watanabe *et al.* (1974)
	18:3 ω3 20% of lipid	Takeuchi &Watanabe (1972 a)
	ω3HUFA 10% of lipid	Takeuchi &Watanabe (1972 a)
Carp	18:2 ω6 1% and	Watanabe *et al.* (1974)
	18:3 ω3 1%	Takeuchi &Watanabe (1977 b)
Eel	18:2 ω6 0.5% and	Takeuchi *et al.* (1980)
	18:3 ω3 0.5%	
Chum salmon	18:2 ω6 1% and	Takeuchi *et al.* (1979, 1980)
	18:3 ω3 1%	
	ω3HUFA 0.5%	Takeuchi *et al.* (1980)
Coho salmon	Tri-18:3 ω3 1–2.5%	Yu & Sinnhuber (1979)
Ayu	18:3 ω3 1% or	Kanazawa *et al.* (1982)
	20:5 ω3 1%	
Tilapia zillii	18:2 ω6 1% or	Kanazawa *et al.* (1980)
	20:4 ω6 1%	
Tilapia nilotica	18:2 ω6 0.5%	Takeuchi *et al.* (1983)
Red sea bream	ω3HUFA 0.5% or	Yone *et al.* (1978)
	20:5 ω3 0.5%	
Turbot	ω3HUFA 0.8%	Gatesoupe *et al.* (1977)
Yellowtail	ω3HUFA 2%	Deshimaru *et al.* (1984)

Rotifers

The relationship between nutritional quality of rotifers as a live food and their culture organism, e.g. baker's yeast or *Chlorella*, was investigated from the viewpoint of EFA for fish (Watanabe *et al.*, 1978 c). Table 2 shows some of the ω3 fatty acids of rotifers cultured with baker's yeast, marine *Chlorella* or both the organisms. The most striking difference was the content of EFA. The rotifers cultured with yeast were quite low in ω3 highly unsaturated fatty acids (ω3HUFA) such as 20:5ω3, and high in monoenoic fatty acids such as 16:1 and 18:1. Those cultured with marine *Chlorella* contained high amount of 20:5ω3, which is one of the EFA for marine fish. The rotifers fed on both yeast and *Chlorella* showed intermediate values. These results may explain why rotifers cultured with yeast are always inferior to those cultured with marine *Chlorella* in their nutritional quality as a live feed.

TABLE 2. *Some ω3 fatty acids in the total lipids from rotifer* Brachionus plicatilis *cultured with baker's yeast* Saccharomyces cerevisiae *and marine* Chlorella

Fatty acid	Rotifer fed on		
	Yeast	Yeast + *Chlorella*	*Chlorella*
20:4 ω3	0.4–0.5	0.4– 0.6	tr – 0.2
20:5 ω3	1.0–1.9	8.1–12.0	22.8–30.5
22:5 ω3	tr –0.3	1.7– 2.9	3.0– 3.8
22:6 ω3	tr –0.5	tr – 0.9	tr – 0.5
$\lesssim$ ω3HUFA*	2.2–3.1	11.3–14.7	26.2–31.9
Lipid in rotifer (%)	1.4–2.3	2.2– 2.8	3.7– 4.2

*$C_{20:3} \lesssim$ ω3 fatty acids (Watanabe, 1985. *Plant and Soil*, **89**: 351-369).

Table 3 lists some fatty acids in the total lipid of the culture organisms. The baker's yeast used for mass culture of rotifers contains a fairly high amount (52–82%) of monoethylenic fatty acids, 16:1 and 18:1, and essentially no ω3HUFA. On the other hand, marine *Chlorella* contains a high level of 20:5ω3. These results show why rotifers cultured with yeast always contain little ω3HUFA. The fatty acid composition of freshwater *Chlorella* is quite different from that of marine *Chlorella*. The freshwater *Chlorella* contains high amount of 18:2ω6 and 18:3ω3, but is low in ω3HUFA. Consequently, rotifers cultured with freshwater *Chlorella* had high levels of 18:2ω6 and 18:3ω3. Thus marine *Chlorella* should be used as the culture organism for rotifers intended as a food for larval marine fish.

These results have shown that the fatty acid composition of rotifers is readily affected by the fatty acids of their food organism. Experiments were then conducted to verify the relationship between the dietary value of rotifers and their ω3HUFA content by feeding them marine *Chlorella* or freshwater *Chlorella* and feeding these rotifers to larval red sea bream. When marine *Chlorella* was used as the culture organism, the low level of ω3HUFA in yeast-fed rotifiers increased in proportion to the length of the culture period with *Chlorella*, due to incorporation of 20:5ω3 from the marine *Chlorella*. The dietary value of the rotifers for red sea bream was also found to be significantly improved by secondary culture with marine *Chlorella* for more than 6hr; but not with freshwater *Chlorella*. Thus, the content of ω3HUFA in the rotifers correlates with their dietary value as feed. The high mortalities frequently observed in various kinds of fish larvae reared on rotifers cultured solely on yeast is due to an EFA deficiency in their food source.

TABLE 3. *Fatty acid composition in total lipids of baker's yeast and marine* chlorella

	Baker's yeast	Marine *chlorella*	Freshwater chlorella
	Saccharomyces cerevisiae	*Chlorella minutissima*	*Chlorella regularis*
14:0	3.1	4.3	0.5
16:0	20.0	22.5	16.9
16:1 ω7	27.2	22.3	2.7
18:0	4.7	1.0	4.1
18:1 ω9	26.1	3.1	3.5
18:2 ω6	10.9	3.4	37.3
18:3 ω3	3.2	0.1	9.1
20:1	0.8	0.1	0.1
20:3 ω3	—	4.7	t
20:4 ω6	—		
20:4 ω3	—	0.1	—
20:5 ω3	—	31.8	0.2
22:5 ω3	—	—	—
22:6 ω3	—	—	—

(Watanabe, 1985. *Plant and Soil*, 89: 351-369).

Recently, a foreign species of micro green alga *Tetraselmis tetrathele*, has been introduced to Japan from Southeast Asia as a substitute for marine *Chlorella*. The size and volume are respectively 10 and 30 times bigger than *Chlorella*, and temperature tolerance is also wider than *Chlorella*, suggesting the suitability as feed organism for rotifers, especially for culture of rotifers in cold water areas. The rotifers cultured with these algae contained a relatively high level of 18:3ω3 which is known to exert ill effect on marine species such as red sea bream, but they also contained about 8–10%

of 20:5ω3 which is one of the EFA for marine fish. However, the recent study on the dietary value of rotifers fed on *Tetraselmis* for various marine fish has demonstrated that the nutritional quality of rotifers cultured with the algae was found to be almost comparable to rotifers fed on marine *Chlorella* in the feeding experiments of about one month duration. But the dietary value was slightly varied for different fish species (Table 4).

TABLE 4. *Dietary value to larval red sea bream of rotifers cultured with* Tetraselmis tetrathele

	Rotifer fed	Total length (mm)	Body weight (mg)	Survival (%)
21 days	*Tetraselmis*	5.8	1.1	95.7
		5.7	1.0	94.7
	Tetraselmis & *Chlorella*	5.8	1.2	95.3
		5.9	1.3	93.4
	Chlorella	5.8	1.1	93.0
		6.0	1.2	93.9
30 days	*Tetraselmis*	8.1	5.2	75.6
		7.5	4.2	83.5
	Tetraselmis & *Chlorella*	8.8	6.6	80.1
		8.7	6.3	68.0
	Chlorella	6.9	5.8	65.1
		6.5	4.7	75.6

Another algal species *Isochrysis galbana* or *Monochrysis* sp., is also frequently utilized as a feed organism for rotifers and *Artemia* nauplii in some geographical locations where marine *Chlorella* is not available. The dietary value of rotifers or *Artemia* nauplii was also reported to be improved by culturing with these algae. This is mainly due to the fatty acid spectrum of *I. galbana* which is in general high in proportion of 20:5ω3 and 22:6ω3, although uptake of ω3HUFA from the algae is dependent upon culture conditions as observed in rotifers cultured with marine *Chlorella*.

As the culture medium for rotifers, alcohol fermentation slops are experimentally introduced. The slops still containing nutrients for growth of brwer's yeast are used for culture of microbial flock consisting of marine yeast and bacteria, and then the microbial flocke are added to culture tank of rotifers. The rotifers cultured by this method are also low in the concentration of ω3HUFA and enrichment is required before feeding them to fish larvae.

Photosynthetic bacteria are also used for culture of rotifers in combination with baker's yeast by continuous recycle system in Japan. However, the rotifers cultured by this method were found

to be very high in 18:2ω6, thus being not recommended to use for larval rearing of marine species (Watanabe *et al.*, 1983).

TABLE 5. *Fatty acid composition, total lipid, polar lipid and triglyceride fractions of* Daphnia *sp.*

Fatty acid	Total lipid	Polar lipid	Triglyceride
16:0	15.3	14.3	17.1
16:1 ω7	12.4	9.6	12.2
18:0	4.8	6.6	6.3
18:1 ω9	12.8	17.7	16.7
18:2 ω6	4.4	4.7	3.9
18:3 ω3	11.0	11.0	6.4
18:4 ω3 & 20:0	2.9	2.0	1.8
20:326 & 20:426	3.6	4.0	5.2
20:4 ω3	0.6	0.4	0.4
20:5 ω3	16.5	16.2	7.5
22:6 ω3	0.2	0.7	—

(Watanabe *et al.*, 1978. *Bull. Japan. Soc. Sci. Fish.*, 44: 1223-1227).

Artemia

The nauplii of *Artemia* have been widely used as a food in the production of juvenile marine fish. However, feeding with *Artemia* alone frequently resulted in high mortalities in various marine fish (Fushini, 1971; Fujita, 1973; Kitajima, 1978), although this phenomenon depended upon the fish species as well as the site of production of *Artemia*. Some species of flounder, one species of mullet *(Liza haemotochiela)*, one salmonid *(Plecoglossus altivelis)* and some gobiid fish are not easily affected, but the larvae of yellowtail *(Seriola quinqueradaita)* are very susceptible to this phenomenon (Fujita, 1972, 1973). In addition, many investigators have reported heavy losses of larval prawns, crabs, and marine fish fed on *Artemia* nauplii from Utah (Little, 1969; Reeve, 1969; Wickens, 1972). The dietary value of *Artemia* nauplii was found to be improved when the nauplii were fed to fish with marine copepods such as *Tigriopus* and *Acartia* (Fukusho, 1974).

As described earlier feeding the fish with yeast fed rotifers resulted in various pathological syndromes, such as a dark body colour, lack of appetite, lethargy and heavy mortalities. The chief cause of these syndromes was found to be an EFA deficiency in the fish (Watanabe *et al.*, 1978 a, 1979). The symptoms observed in red sea bream were very similar to those induced by *Artemia* nauplii. At first Watanabe *et al.* (1980) analysed fatty acid compositions of *Artemia* eggs and nauplii from different geographical

TABLE 6. *Certain fatty acids of total lipids from baker's yeast, the yeast supplemented with cuttlefish liver oil (ω - yeast) and rotifers cultured with these yeasts.*

Fatty acid	Baker's yeast	ω-yeast	Rotifers cultured	
			Baker's yeast	ω-yeast
16:0	8.3–20.0	13.4–16.9	6– 7	10–12
16:1 ω7	14.2–38.2	5.0– 6.6	26–27	10–11
18:0	3.4– 8.4	2.3– 2.6	3– 4	2– 3
18:1 ω9	26.1–43.9	15.5–16.4	26–30	22–24
18:2 ω6	2.8–15.1	1.0– 1.1	7– 9	2– 4
18:3 ω3	0.5– 6.4	0.8– 0.9		0.7–0.8
20:1	tr – 1.6	8.4– 9.2	3– 4	8–10
20:3 ω3		3.0– 3.4	1– 2	3– 4
20:4 ω6				
20:5 ω3		**13.4–17.4**	**1– 2**	**9–12**
22:5 ω3		0.9– 1.4	0–0.4	2– 3
22:6 ω3		**12.8–15.6**		**7– 9**
Σ ω3HUFA		33.5–35.8		25–26
Lipid %	1.0– 1.6	12.3–15.6	1.4–1.9	3.3–5.4

(Watanabe *et al.*, 1983. *Aquaculture*, 34: 115-143)

locations and found that *Artemia* could be classified into two types according to the fatty acid compositions. As shown in Fig. 1, one (the freshwater type) contained a high concentration of 18:3ω3, which is an EFA for freshwater fish, and the other (the marine type) was high in 20:5ω3, which is one of the EFA for marine fish. Furthermore, Artemia of "The marine type" were found to be a satisfactory food for juvenile red sea bream, and the dietary value of the nauplii was improved by feeding them marine *Chlorella* and ω-yeast, both containing substantial amounts of the EFA required by marine fish. These results suggest that the class of EFA contained in *Artemia* is the principal factor in the variation in food value of *Artemia* to fish, as demonstrated in the case of rotifers. The difference in susceptibility of larval fish from species to species to *Artemia* nauplii in terms of mortality and various syndromes were also due to the difference in EFA requirements of fish as shown in Table 1. Wickens (1972) reported that the food value of *Artemia* from Utah was improved by allowing them to feed on *Isochrysis galbana*. This may be due to the incorporation of ω3HUFA from *I. galbana*, which generally contained high amounts of 20:5ω3 and 22:6ω3 (Watanabe and Ackman, 1974). Moreover, the food value of *Artemia* is effectively improved when they are fed to fish together with *Tigriopus* or *Acartia* (Fukusho, 1974), both rich in 20:5ω3 and 22:6ω3 (Watanabe *et al.*, 1980).

As shown in Fig.1, *Artemia* from San Francisco are, in general, high in concentration of 18:3ω3, although the concentration largely fluctuated from year to year or lot to lot, even in the cysts collected within the same year at the same locations. But they generally

belong to "the freshwater type". On the other hand, *Artemia* from China are low in the content of 18:3ω3 and high in 20:5ω3, and classified into "the marine type". According to the data available at present, *Artemia* which are classified into "the marine type" are those from China, Australia, Italy, Israel and Thailand.

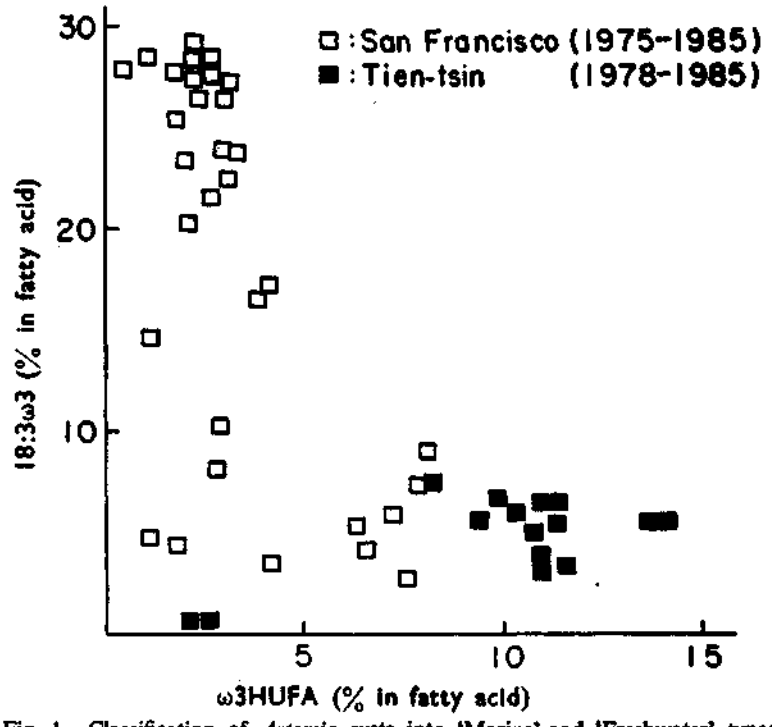


Fig. 1. Classification of *Artemia* cysts into 'Marine' and 'Freshwater' types based on the proportions of their 18:3ω3 and ω3HUFA.

The cysts from Brazil are characteristically low in the content of 18:3ω3 and high in the proportion of ω6 fatty acids such as 18:2ω6 and 20:4ω6, as shown in Fig. 2. The Brazil cysts originally came from California and should have the same fatty acid patterns as those of San Francisco. However, their fatty acid spectra were quite different from those of San Francisco. These differences in fatty acid composition due to geographical strains may be dependent upon differences of algal blooms in the environmental waters where adult *Artemia* take algae as food for their recycles. Changes in the content of algal blooms in terms of microalgal species due to weather and environmental conditions may result in some seasonal variation in fatty acid composition of *Artemia* cysts.

TABLE 7. *Comparison of growth and survival rate of larval red sea bream fed on the rotifers cultured with yeast & ω-Yeast respectively*

	Rotifer used	No. of fish	Total length at final (mm)	Rate of survival (%)	Survival at activity test (%)
Expt. I	ω-Yeast	30,000	9.28±0.77	73.5	86.0
	Yeast	„	7.10±0.78	13.0	12.5
Expt. II	ω-Yeast	15,000	10.11±0.87	76.2	92.9
	Chlorella	„	10.21±1.60	57.1	91.7
	Y 12h C	„	9.11±1.24	27.9	93.2
	ω-Yeast	24,000	10.32±1.28	76.9	92.5
Expt. III	Chlorella	„	9.78	70.1	91.5
	Y 3h C	„	8.85±1.09	27.6	55.8
Exp. IV*	ω-Yeast	10,000	10.91±0.94	68.9	95.5
	Yeast	„	6.24±0.62	3.2	45.9

*Black sea bream (Watanabe *et al.*, 1983. *Aquaculture*, 34: 115-143).

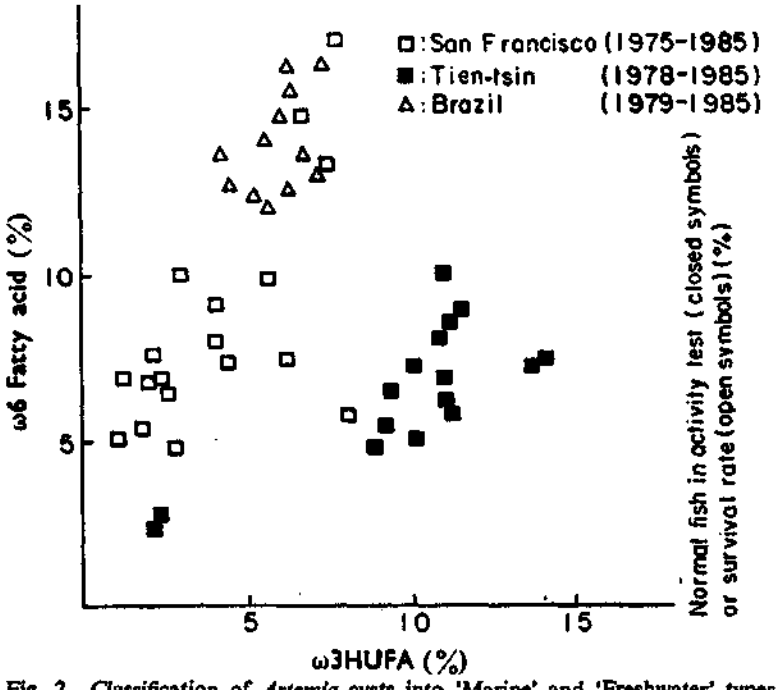


Fig. 2. Classification of *Artemia* cysts into 'Marine' and 'Freshwater' types based on the proportions of their ω6 fatty acids and ω3HUFA.

Artemia of "the marine type" are usually satisfactory as a food for any kinds of fish species and shrimps, although it depends upon the total concentration of ω3HUFA in the nauplii. Various trials have been conducted in order to improve the dietary value for marine fish juveniles of *Artemia* nauplii of "the freshwater type" by allowing them to feed on ω3HUFA. These improvements were also effected mainly by the same methods as for rotifers, the direct and indirect methods.

Other live feeds

The marine copepods *Tigriopus* and *Acartia* and the freshwater *Moina* and *Daphnia* are also well known to be suitable as feeds for rearing juvenile fish of about 7 mm in body length. Watanabe *et al.* (1978 c) analysed fatty acids of these zooplankters. *Tigriopus* was found to contain relatively high amounts of 20:5ω3 and 22:6ω3, irrespective of culture media and organisms, such as backer's yeast and soy sauce cake; this result suggests a high nutritional value for fish. On the other hand, the content of ω3HUFA in the lipid of *Moina* was significantly affected by the culture organisms, as observed in rotifers. *Moina* cultured with yeast contained high levels of monoethylenic fatty acids and low levels of ω3HUFA, while those cultured with poultry manure had high contents of 20:5ω3, indicating that the former is inferior to the latter as a live-feed for fish. *Moina* was also found to take up emulsified lipids very easily by the direct method which is described in the next chapter. *Acartia* collected in the sea was found to be a very good food for marine fish; it contained both 20:5ω3 and 22:6ω3 at fairly high levels, making a total ω3HUFA of 30–60%, although some seasonal variation in ω3HUFA was apparent. The fatty acid spectrum of *Daphnia* (Table 5) also makes it a suitable food for fish from the view point of EFA.

3

ENRICHMENT OF LIVE FEEDS

After finding the importance of ω3HUFA in live feeds, various trials have been conducted to improve their nutritional quality by feeding them with various kinds of materials such as microdiets, microencapsulated diets, a new type of baker's yeast and emulsified lipids rich in ω3HUFA together with fat-soluble vitamins. Among them the so-called direct method in which emulsified lipids are used and the indirect method using a newly developed yeast are at present most popular in Japan. Thus in this, these two methods are introduced, although the indirect method with the new type of yeast is not available in all the regions.

Indirect methods

Based upon the results that baker's yeast contains a fairly high amount of monethylenic fatty acids and no ω3HUFA, and that fatty acid composition of rotifers is readily affected by the fatty acids of the culture organisms, a new kind of yeast has been developed as a culture organism for rotifers in order to improve upon the nutritional value for fish larvae of rotifers cultured on baker's yeast (Imada *et al.*, 1979). This new type of yeast (designated as ω-yeast) was produced by adding fish oil or cuttlefish liver oil as a supplement to the culture medium of baker's yeast, resulting in a high content of lipid and ω3HUFA, the EFA for both marine and freshwater fish species (Table 6). The rotifers cultured with ω-yeast were high in lipid content in general, together with ωHUFA, as a result of the oils added to the backer's yeast. The incorporation of ω3HUFA from ω-yeast reached a maximum at around 12 hr of feeding. Furthermore; the dietary value of the rotifers to fish larvae was significantly improved, compared to that of rotifers cultured on marine *Chlorella* as shown in Table 7 (Kitajima *et al.*, 1980 a, 1980 b; Oka *et al.*, 1980). These results clearly indicate that rotifers grown on the newly developed yeast have a superior food value to those grown on the original yeast. Arakawa *et al.* (1979) also found that rotifers grown on ω-yeast had a superior

food value for larval pufferfish *Fugu rubripes*. Recently, Fukusho *et al.* (1980) mass cultured *Tigriopus* with ω-yeast and found that its nutritional quality for mud dab was much enhanced. This ω-yeast was also found to be effective for the improvement of dietary value of other live foods such as *Artemia* nauplii and *Moina*.

However, it is very important to keep in mind that incorporation of ω3HUFA together with lipid into live feeds is sometimes largely fluctuated due to culture conditions in terms of water temperature,

TABLE 8. *Improvement of dietary value of* Artemia *nauplii for fish larvae by both the direct and indirect methods*

Feed given to *Artemia*	ω3 HUFA in *Artemia* (%)	Total body length (mm) Initial	Final	Total body weight (mg) Initial	Final	Survival rate (%)	Normal fish in activity test (%)*
Red sea bream (9 days feeding)							
Baker's yeast	0.12	14.7	22.0	35.2	151.9	58.9	23.0
Corn oil	0.03	„	22.6	„	158.0	52.3	31.5
Pollock liver oil	0.21	„	23.7	„	188.9	76.3	86.5
Cuttlefish liver oil	0.77	„	23.6	„	182.5	83.1	99.6
ω3HUFA concentrate	0.71	„	23.4	„	178.7	72.0	99.3
Red sea bream (7 days feeding)							
Baker's yeast	0.9	8.9	12.5			32.0	21.4
Corn oil	0.07	„	12.2			35.7	15.2
Pollock liver oil	0.15	„	13.4			39.7	56.7
Pollock & Cuttlefish liver oil (1:1)	0.30	„	13.1			53.4	67.9
Cuttlefish liver oil	0.33	„	13.3			63.1	98.2
ω3HUFA concentrate	1.01	„	14.1			52.0	96.7
Rock sea bream (10 days feeding)							
ω-Yeast	0.30	9.7	20.4	9.0	145.1	78.3	86.7
Cuttlefish liver oil	0.31	„	20.3	„	142.9	81.4	100
Baker's yeast	0.08	„	19.3	„	117.2	41.4	3.4
Control**	0.10	„	19.5	„	124.8	59.2	10.0
Japanese flounder (19 days feeding)							
Cuttlefish liver oil	0.40	7.4	12.4		13.7	67.6	80.0
Corn oil	0.05	7.2	9.9		5.8	27.1	0
Control*	0.05	7.3	11.2		9.8	35.6	13.3

* 30–50 fish were removed from water with a scoop net, held for 5 hr and moved to a 30 l tank for a check of fish activity.

** Nauplii just after hatching (48 hr).

density and activity of live feeds, feeding rate of ω-yeast, etc. Furthermore, a high amount of lipid contained in ω-yeast also sometimes resulted in some mortality in rotifers and *Artemia* nauplii and

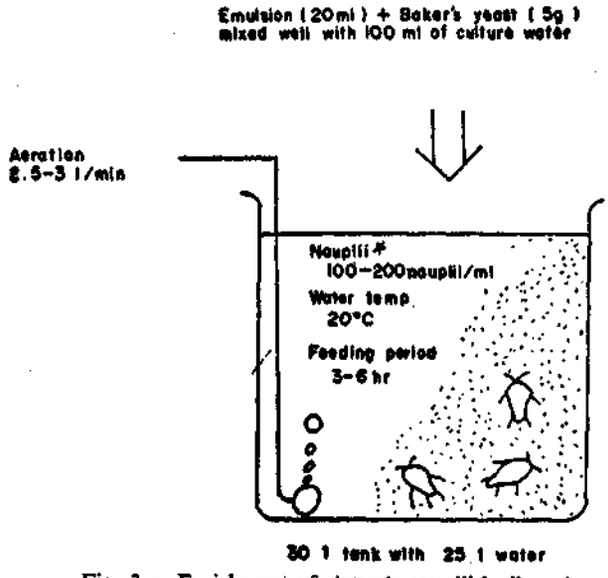


Fig. 3 a. Enrichment of *Artemia* nauplii (collected at 40 hr after incubation or those passed more than 10 hr after hatching (26–28°C)].

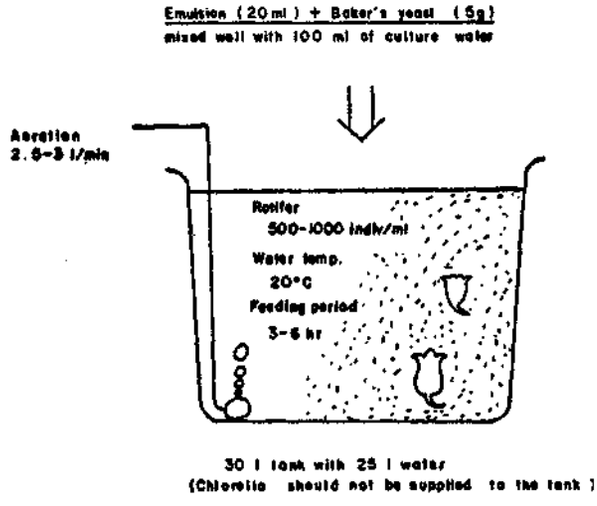


Fig. 3 b. Enrichment of rotifer.

Moina. This method may be called the indirect method, because life feeds are enriched with ω3HUFA through the yeast in comparison with the direct method where they are directly fed on ω3HUFA together with lipid. The availability of ω-yeast is unfortunately limited at present, thus the second method, the direct method which can be applied in any place, is recommended to enrich the dietary value of rotifers and *Artemia* nauplii.

Direct method

This method has been developed by Watanabe *et al.* (1982). In this direct method, the lipids containing ω3HUFA were homogenized with a small amount of raw egg yolk and water and resulting emulsion was fed directly to live feeds such as rotifers and *Artemia* nauplii together with baker's yeast (Fig. 3 a, b). As shown in Fig. 4, both rotifers and *Artemia* nauplii took up lipids very easily and the concentration of ω3HUFA reached a maximum between 6 and 12 hr of feeding, as observed in the indirect method. The two methods, direct and indirect, are also found to be very effective for improving the dietary value of other live feeds.

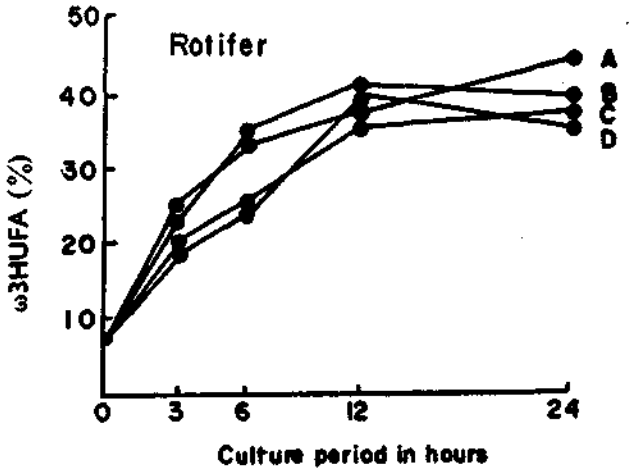


Fig. 4 a. Incorporation of lipids emulsified with various reagents (A, B, C and D) in rotifers by the direct method.

The dietary value of rotifers and *Artemia* nauplii to fish larvae was improved by incorporating ω3HUFA from emulsified lipids, and was proportional to the ω3HUFA content in the live feeds. Table 8 shows the results of improvement of dietary value of *Artemia* nauplii of "the freshwater type" to larval marine fish of some species. In each case feeding the newly hatched nauplii containing low amounts of ω3HUFA resulted in lower growth rate and survival,

and especially the percentage of normal fish in activity test was significantly lower than those fed on the nauplii enriched with emulsified lipids. Elevation of ω3HUFA levels in the nauplii by allowing them to feed emulsified cuttlefish liver oil, pollock liver oil or the ω3HUFA concentrate effectively improved these conditions. On the other hand, the dietary value of the nauplii fed on baker's yeast alone or on corn oil, containing few ω3HUFA, was very low for marine fish. Although any type of *Artemia* may be satisfactory for freshwater fish judging from their EFA requirement (Table 1), it is necessary to check the fatty acid composition of *Artemia* for use as a food for marine fish. If its fatty acid composition is not known, the *Artemia* should be fed to fish together with other marine copepods or should be fed on lipids containing ω3HUFA to prevent heavy fish losses from various syndromes.

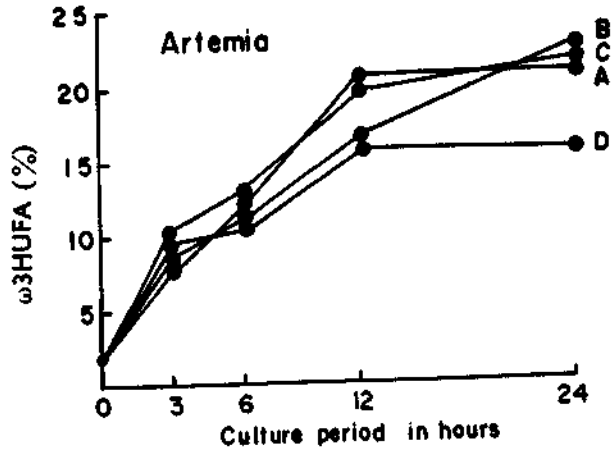


Fig. 4 b. Incorporation of lipids emulsified with various reagents (A, B, C and D) in *Artemia* nauplii by the direct method.

TABLE 9. *The concentration of* ω6 *and* ω3 *fatty acids in some lipids (%)*

Lipid source	ω6	ω3	
		18:ω3	3 ωHUFA*
Pollock liver oil	2.0–3.5	0.2–2.0	12–20
Cod liver oil	2.0–3.5	1.0–1.5	20–25
Squid liver oil	2.0–4.0	1.0–1.5	25–30
Herring oil	1.5–2.5	0.5–1.0	11–15
Sardine oil	2.0–4.0	1.0–2.0	20–25
Bonito oil	2.5–4.5	1.0–2.0	20–30
Soybean oil	49–52	1.5–11.0	—
Corn oil	34–62	0–3.0	—
Cotton seed oil	34–55	—	—
Olive oil	5–8	0.5–1.5	—
Safflower oil	39–79	0.04–6.0	—

* 20:3 ω3 $\leq$ ω3 fatty acids, mainly consists of 20:5 ω3 and 22:6 ω3.

Fig. 5 shows the relationship between the ω3HUFA content of *Artemia* nauplii and fish activity of survival rate, prepared based upon the results of the feeding experiment with red sea bream, rock sea bream and Japanese flounder. The nauplii containing ω3HUFA at levels of 0.3-0.5% may be satisfactory as a single feed for most of fish species. This requirement of nauplii for ω3HUFA to satisfy the EFA requirement of larval marine fish was found to be almost the same as that in case of rotifers.

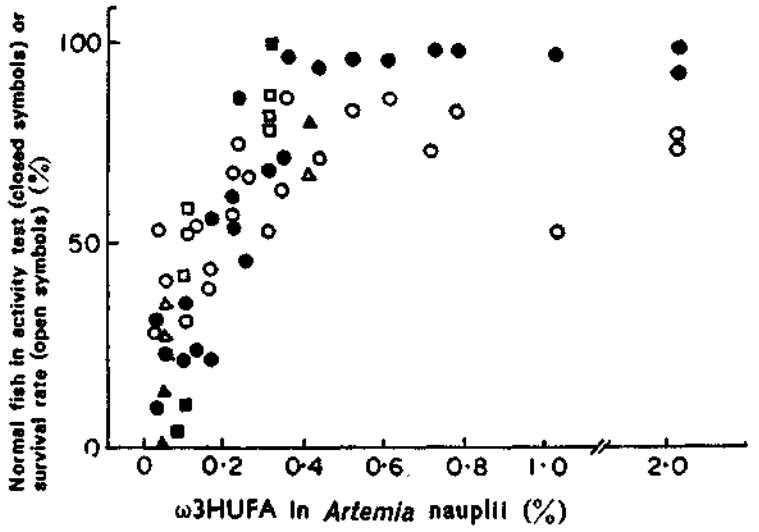


Fig. 5. Relationship between the ω3HUFA content of *Artemia* nauplii and fish activity and survival rate. The data are from red sea bream (circles), rock sea bream (squares) and Japanese flounder (triangles).

By using both methods, the direct and indirect, it was found possible to further improve the dietary value of live feeds by allowing them to take up from culture medium not only ω3HUFA, but also fat-soluble vitamins together with lipids (Watanabe *et al.*, 1982). Figs. 6 and 7 show the effect of *Artemia* nauplii biomass and water temperature on the incorporation of vitamin A and α-tocopherol. Both vitamins A and E were found to be incorporated into the nauplii as observed in rotifers, and the incorporation was highest when the nauplii density was lowest at the water temperature of 21°C. Fig. 8 shows the effect of *Artemia* age past incubation and duration of enrichment on the incorporation of α-tocopherol, 40 hr, 52 hr and 64 hr in the figure indicating hours after egg incubation. In each case a maximum incorporation was obtained after about 6 hr of enrichment, and was highest in the nauplii collected at 52 hr after incubation.

a. Preparation of emulsified lipids

For the preparation of emulsified lipids, the so-called 'Mayonnaise', lipids must be high in the concentration of ω3HUFA. Some locally availabe lipids high in the ω3HUFA content are listed in Table 9. As shown in Fig. 4 a in ordinary preparation about 5 g of fish oil is homogenized for 2-3 min by homogenizer or juicer mixer or by shaking vigorously. The resulting emulsion should be studied by microscope or overhead projector to check homogenous condition in terms of particle size, and can be kept in a refrigerator until

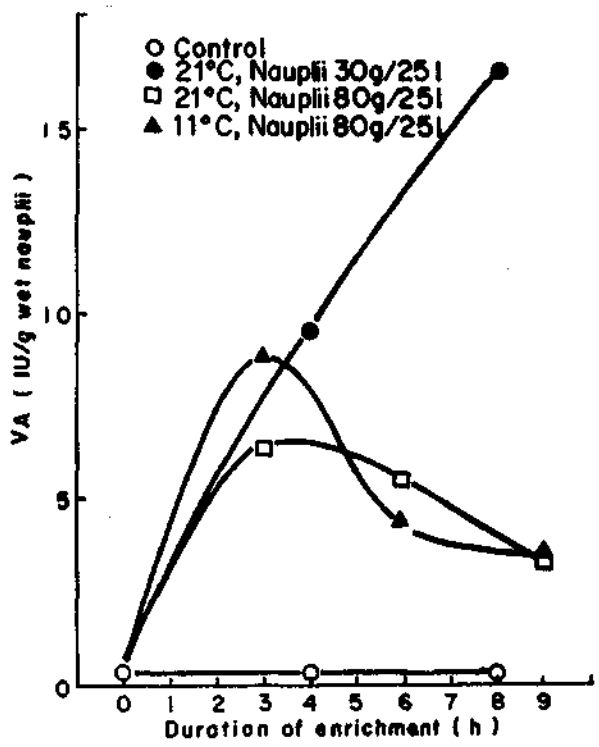


Fig. 6. The effect of *Artemia* nauplii biomass and water temperature on the incorporation of vitamin A.

needed, although it is necessary to shake again vigorously before feeding them to live feeds, because the emulsion may be separated into water and lipid layers during storage. If required, further enrichment can be achieved by supplementing both water and fat-soluble vitamins to the emulsion before homogenisation. At present various types of emulsifiers are also available for preparation of emulsion.

b. Hatching of nauplii

The conditions of hatching *Artemia* cysts and obtaining the nauplii foı enrichment are described as below.

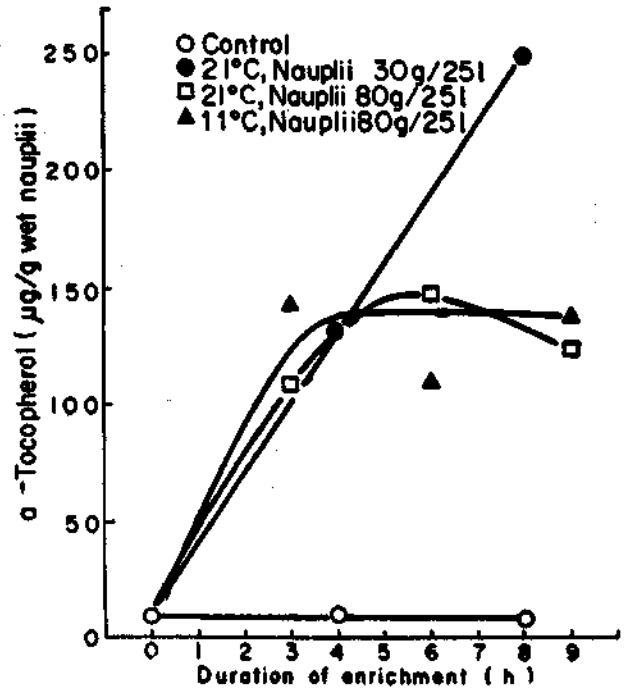


Fig. 7. The effect of *Artemia* nauplii biomass and water temperature on the incorporation of α - tocopherol.

Artemia HATCHING CONDITIONS

Incubation temperature: 28°C (warmed up with a 200W heater)
Salinity: 29.5‰ (12.7°C)
Duration: Collected at 45 hrs. after incubation.
Light: Natural condition, 5,000 lux at daytime.
Aeration: 3.000 ml/min.
Tank: A 20 litres polycarbonate tank with black colour.

c. Enrichment

Fig. 3 a, b shows one example of conditions for feeding emulsion to rotifers and *Artemia* nauplii. The incorporation of lipids together

with ω3HUFA in rotifers and nauplii is in general greatly affected by enrichment conditions in terms of lipid content of emulsion and type of lipid. The amount of emulsified lipid supplied to the culture medium depends upon population density of live feeds, their feeding activity and water temperature, etc. Conditions suitable for enrichment of rotifers and *Artemia* nauplii should be surveyed according to each situation.

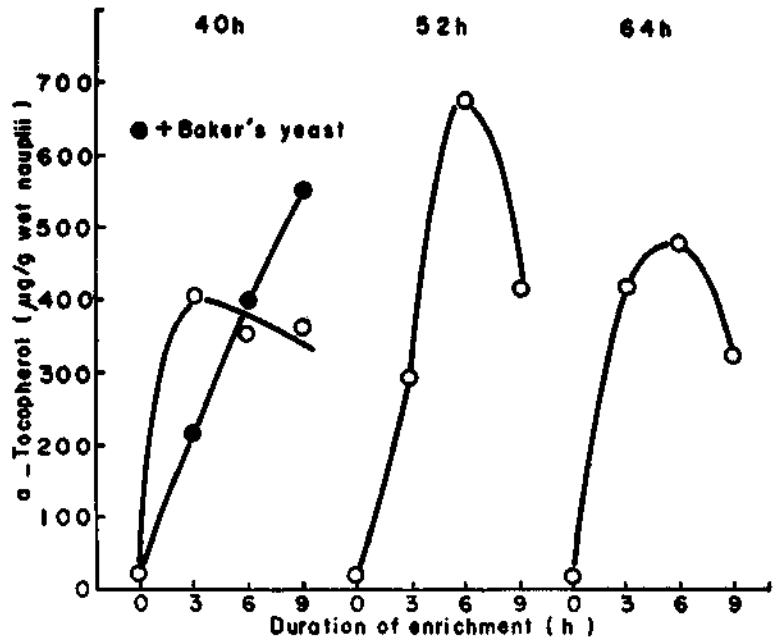


Fig. 8. The effect of *Artemia* age past incubation and duration of enrichment on the incorporation of ∝ - tocopherol. 40 h, 52 h and 64 h in the figure are hours after egg incubation.

d. Feeding enriched live feeds to larval fish

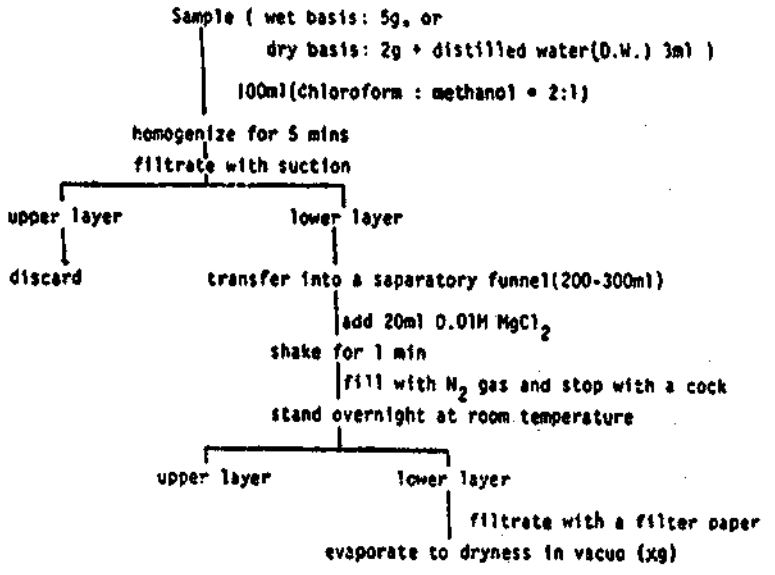
The count of number of live feeds in a culture medium should be ncessary before feeding them to larval fish. Because this treatment sometimes results in some mortality of live feeds in the enrichment tank. The rotifers or the nauplii are harvested by using a micronet of about 69 μ and then they are washed with sea water before feeding.

e. Collection of analytical sample

Sample collection of live feeds from the culture medium for chemical analysis should be done before enrichment (the initial

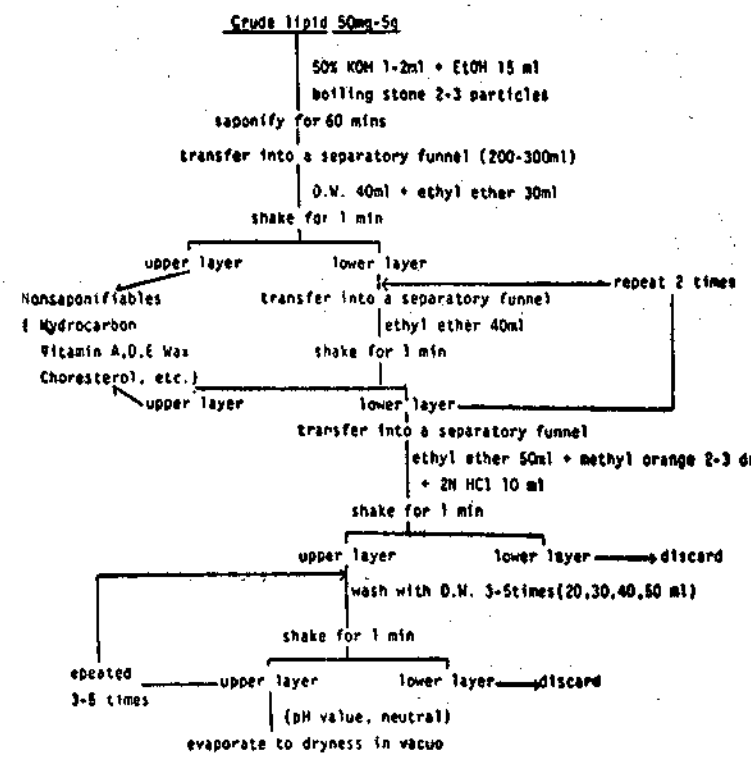
sample) and at 3, 6, 12 and 24 hr after enrichment. Before sample collection various dust formed by mixture of dead organisms and oil must be removed by using a micronet. A high amount of oil containing vitamins are frequently seen to be attached to the surface of dead rotifers and nauplii. Samples collected by the net are washed thoroughly with tap water for 5 min and then followed by distilled water. Water in the surface of live feeds are wiped out with filter paper, weighed and kept at -20°C until analysis. The fatty acid composition of the samples is analysed by gas chromatography according to the scheme given below.

EXTRACTION OF CRUDE LIPIDS



$$\text{Crude lipid } \% = x/5\ 100$$

Preparation of fatty acid mixtures



Preparation of Emulsified Lipid

Fish oil with fat-soluble vitamins (5 g)

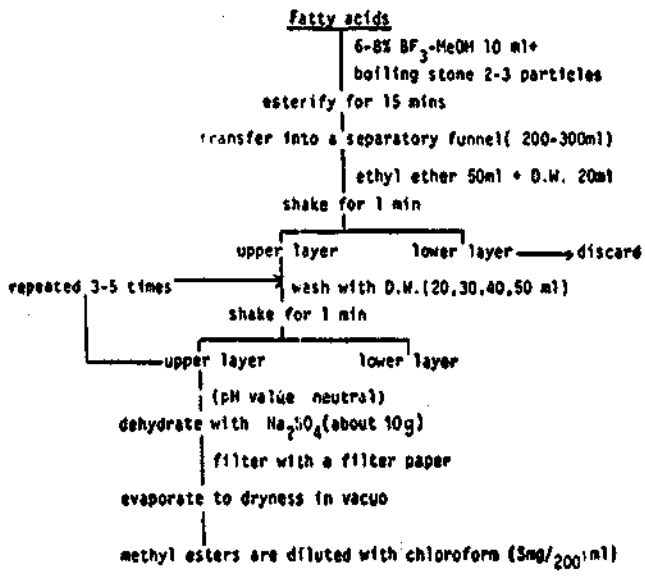
- Water soluble vitamins (10 g)
- Raw egg yolk (1 g)
- H_2O (100 ml)

Homogenate for 2–3 min by mixer of shaking vigorously.

Emulsion
(Check homogeneous condition of emulsion by microscope)

Keep in a refrigerator until use

Preparation of methyl ester



The resulting solutions (0.5ul) are injected into a gas chromatograph

Crude lipid % = x/5 × 100

GLC Operating Conditions

Apparatus	:	Shimadzu Gas Chromatograph GC-7A or GC-7AG with a hydrogen flame ionization detector
Column	:	Glass tube 2.0 m x 3 mm Ø
Packing	:	5% Shinchrom E-71 on 80–100 mesh Shimalite
Carrier gas	:	Nitrogen
Flow rate		
Nitrogen	:	2.2 Kg/cm²
Hydrogen	:	0.7 Kg/cm²
Air	:	0.6 Kg/cm²
Temperature		
Detector	:	250°C
Injection port	:	250°C
Column	:	Isothermal 210°C

CONCLUSION

Live food organisms are still extensively used for rearing larval fish in aquaculture. The nutritional quality of these food organisms very much depends upon the conditions of their culture. Very often high mortalities of fish larvae were encountered by feeding live food organisms deficient in essential fatty acids (EFA). Lipid enrichment techniques described here were found to be very effective and can be successfully employed to enhance the EFA content of a variety of live food organisms.

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