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# International Study on *Artemia*[1] XXXV. Techniques to manipulate the fatty acid profile in *Artemia* nauplii, and the effect on its nutritional effectiveness for the marine crustacean *Mysidopsis bahia* (M.).

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**Abstract**

The importance of essential fatty acids for the early larval stages of cultured marine fish and crustacean larvae is well documented. In this regard, the variability in essential fatty acid content of their live prey *Artemia* is critical.

This paper describes different techniques for improving the fatty acid profile in *Artemia* nauplii using microparticles and emulsions as enrichment diets. These diets can be applied during the incubation of the cysts and/or after separation of the nauplii. After enrichment, the nauplii not only contain high levels of the essential fatty acid 20 : 5ω3 (up to 13.5% or 35.2 mg/g) but also considerable levels of 22:6ω3 (up to 7% or 18.1 mg/g), another essential fatty acid which is rarely found in *Artemia.* Fatty acid enrichment of *Artemia* nauplii therefore not only minimizes differences in nutritional quality between strains (*e.g.* in 20:5ω3 content), but it also converts the nauplii into a high-quality food (*e.g.* 22:6ω3).

The beneficial effect of using essential fatty acid enriched *Artemia* as a larval diet is demonstrated in a bioassay test with *Mysidopsis bahia* (M.).

## Introduction

In the last decade, the nutritional value of *Artemia* as a food source for larval fish and crustaceans has been very much on the foreground. Indeed, using different sources of *Artemia* or even different batches from the same source, an important variation in nutritional effectiveness for marine larval organisms has been experienced (reviews in Léger *et al.*, 1986, 1987). This geographical and temporal variation has generated intensive research in determining which factors govern the nutritional value of *Artemia.* Besides differences in naupliar dimensions, one *Artemia* factor has unambiguously been related to the culture success in marine larviculture : *i.e.* the presence of essential fatty acids, especially the long chain highly unsaturated fatty acids (HUFA) such as eicosapentaenoic acid (20:5ω3).

[1] International Interdisciplinary Study on *Artemia* strains coordinated by the Artemia Reference Center, State University of Ghent, Belgium.

When comparing various strains and batches of *Artemia* (Table I) a considerable variability in 20:5ω3 is noticed. Striking is the wide range in 20:5ω3 content in San Francisco Bay, Brazil, and China *Artemia* and the narrow range in Utah *Artemia*. Ecological differences between evaporation ponds (*e.g.* salinity, nutrient composition) in a solar saltworks (*e.g.* San Francisco Bay, Brazil, China) are indeed translated in differences in food composition (*e.g.* phytoplankton) *i.e.* in a varying fatty acid profile of the *Artemia* diet, which in turn influences the fatty acid quality of the offspring (Lavens *et al.*, 1987). Ecologically stable environments such as hypersaline lakes (*e.g.* Great Salt Lake and Chaplin Lake) will therefore produce cysts with a more predictable fatty acid profile (Léger *et al.*, 1986). Docosahexaenoic acid (22:6ω3), another essential fatty acid of the marine type (Yone, 1978 ; Holland and Jones, 1981 ; Léger and Frémont, 1981 ; Léger *et al.*, 1985 ; Bell *et al.*, 1985 ; Jones *et al.*, in press) has been detected in insignificant levels only in *Artemia* nauplii (Léger *et al.*, 1986). The unpredictability of essential fatty acid levels and the fact that these essential nutrients may even be completely lacking in *Artemia* are a major constraint for the successful use of *Artemia* as a nutritionally suitable food source in marine larviculture.

TABLE I

Intra-strain variability of 20:5ω3 content in *Artemia*
Data represent the range (area percent) and coefficient of variation of data as compiled by Léger *et al.*, 1986, 1987

| *Artemia* geographical strain | 20:5ω3 range (area %) | coefficient of variation (%) |
|---|---|---|
| USA – California : San Francisco Bay | 0.3-13.3 | 78.6 |
| USA – Utah Great Salt Lake (south arm) | 2.7- 3.6 | 11.8 |
| USA – Utah Great Salt Lake (north arm) | 0.3- 0.4 | 21.2 |
| Canada-Chaplin Lake | 5.2- 9.5 | 18.3 |
| Brazil-Macau | 3.5-10.6 | 43.2 |
| PR China-Tientsin | 1.3-15.4 | 50.5 |

Recently, however, several authors have elaborated techniques for the nutritional enhancement of *Artemia* nauplii (review in Léger *et al.*, 1986). Several types of enrichment diets have been used such as microalgae (Morris, 1956 ; Forster and Wickins, 1967 ; Wickins, 1976 ; Kelly *et al.*, 1977 ; Bromley, 1978 ; Howell, 1979 ; Howell *et al.*, 1981), ω-yeast (Watanabe *et al.* 1978, 1980, 1982, 1983), microparticles and emulsions (Robin, 1982 ; Robin *et al.*, 1981, 1984 ; Gatesoupe, 1982 ; Watanabe *et al.*, 1982 ; Léger *et al.*, 1985). The use of algae for *Artemia* enrichment is not to be recommended since they have to be cultured and contain variable levels of 20:5ω3 and 22:6ω3, the latter being rarely present (Moal *et al.*, 1978 ; Scott and Middleton, 1979 ; Enright, 1984). The use of ω-yeast – baker's yeast enriched with fish-oil, may eliminate the problem of inconsistent levels of the essential fatty acids but has the disadvantage not to be commercially available since the ω-yeast should be used in a living condition (Watanabe, pers. commun). The application of formulated and emulsified diets based on oils containing high levels of HUFA's or HUFA-concentrates provides better opportunities for effective large scale *Artemia* enrichment. Efficient enrichment using such diets will, however, largely depend on diet stability, availability in the water column and on enrichment procedures.

In this paper we describe the use of microparticulate and emulsified HUFA-boosters for *Artemia* nauplii. Culture tests were run with the mysid shrimp *Mysidopsis bahia* in order to evaluate the effect of enrichment on the nutritional value of the *Artemia.* The experiments with microparticles are discussed separately since they were the epochal precursor of the experiments using emulsified diets.

## Materials and methods

### *ARTEMIA* HATCHING

The *Artemia* used for these studies originated from San Pablo Bay (CA-USA batch 1628 ; abbreviated SPB), San Francisco Bay (CA-USA batch 236-2016 ; abbreviated SFB), Great Salt Lake (UT-USA northern arm ; abbreviated GSL) and Tientsin (PR China ; abbreviated TTS). Hatching was carried out in filtered (5 µm) artificial seawater (formula of Owens, in Sorgeloos *et al.*, 1983) at 25 °C or 28 °C, 35 ‰ S, 2 000 lux under continuous aeration. The nauplii were harvested after 24 h (SPB, SFB, GSL) or 48 h (TTS), separated from hatching debris, and thoroughly rinsed before being transferred to the enrichment medium.

### *ARTEMIA* ENRICHMENT

#### *With microparticles*

In a series of experiments carried out in 1981 three experimental microparticulate diets were used : cod liver oil coated ricebran (CLORB), rice oil coated ricebran (RORB), and AA18, an experimental microparticulate diet manufactured by Artemia Systems NV-SA, Gent, Belgium. The diets differed in content of ω3-HUFA (Table II) and were used to study the manipulation of the fatty acid profile in *Artemia* and its effect on the nutritional value for *Mysidopsis bahia.* For this the freshly-hatched SPB (24 h, 25 °C) *Artemia* nauplii were transferred (naupliar density 25/ml) into an aerated suspension of freshly-prepared enrichment diet (0.6 g/l) in filtered (0.2 µm) artificial seawater (35 ‰ S, 25 °C). A turbidity of 15 cm was installed (± 0.6 g diet/l) and maintained. After 24 h the enriched metanauplii were harvested, rinsed, and fed to the mysid larvae or used for analytical examination.

#### *With emulsions*

These enrichment procedures described above were adapted for large scale application of *Artemia* enrichment in fish and shrimp farming (*i.e.* selection of widely available and cheap cysts, of Great Salt Lake, Utah-USA origin, *Artemia* densities up to 300 nauplii/ml during enrichment). Enrichment was performed with the commercial product Selco (Artemia Systems NV-SA, Gent, Belgium), *i.e.* a stable (chemically and physically) enrichment diet under the form of a HUFA enrichment concentrate ; this diet is a self dispersing liquid producing finely dispersed globules (approximately 2 µm) which are readily ingested by *Artemia* nauplii.

*Three enrichment procedures were tested :*

1. Enrichment of freshly-hatched nauplii (separated from their hatching debris after 24 h, 28 °C) incubated at a naupliar density of 300/ml in a prepared emulsion of 0.6 g Selco/l artificial

seawater. The enrichment diet was administered in one, two or three rations. The required weight of Selco was vigorously shaken in water and diluted in the required volume of seawater. The enriched metanauplii were harvested after 12 h, 24 h, or 48 h incubation at 28 °C, and were thoroughly rinsed before feeding or analysis.

2. Enrichment in the hatching medium without separation of the nauplii. A series of trials was set up adding Selco after 24 h hatching incubation at 28 °C without separating the nauplii from the empty cyst shells. For this, disinfected cysts (20 min incubation in 200 ppm active chlorine) were incubated at a density of 2 g/l which yielded approximately 300 nauplii/ml. Enriched metanauplii were harvested and separated from the hatching debris after 12 h, 24 h, or 48 h incubation and thoroughly rinsed before feeding or analysis.

3. Enrichment during hatching incubation using the commercial formulation Supar (Artemia Systems, NV-SA, Gent, Belgium), consisting of *Artemia* cysts treated with a special self emulsifying ω3-HUFA concentrate. Supar cysts were incubated at 2 g/l during 36 h at 28 °C in filtered (0.2 µm) artificial seawater after which the enriched metanauplii were harvested, separated from their hatching debris, and thoroughly rinsed before feeding or analysis.

The effect of temperature (25 °C and 30 °C) and light on ω3-HUFA accumulation in *Artemia* was also investigated in a separate experiment using Selco as enrichment diet.

All enrichment trials were carried out in triplicate in 100 l polyethylene cylindroconical tanks provided with three aeration lines (one open tube and two equipped with air stones) as to maintain 4-5 ppm oxygen during enrichment. One trial with Chinese cysts was carried out in a 2 000 l cylindroconical polyester tank.

### *Mysidopsis bahia* culture test

The nutritional value of freshly-hatched and enriched *Artemia* nauplii was assessed in a bioassay test with juvenile *Mysidopsis bahia* (Molenock). The testing procedure used, is outlined by Léger *et al.* (1987).

### Fatty acid analysis

Fatty acid profiles were determined by capillary gas chromatography. For this, samples were first homogenized with an ultrasonic homogenizer (Sonifier B12), total lipids were extracted according to the method of Bligh and Dyer (1959), and saponification and esterification was done according to the procedure described by Schauer and Simpson (1978). Fatty acid methyl esters were injected on a capillary column (25 m fused silica inner diameter i.d. : 0.32 mm, liquid phase : Silar 10C, film thickness : 0.3 µm) installed in a Carlo Erba Mega 2350 gas chromatograph.

Operating conditions were as follows : on-column injector, carrier gas : hydrogen, flow rate : 2 ml/min, FID detection, oven temperature programme : 105 °C to 150 °C at 10 °C/min and 150 °C to 200 °C at 5 °C/min. Peak identification and quantification was done with a calibrated plotter integrator (HP3390A) and reference standards. Fatty acid composition was expressed in area percent and mg fatty acid methyl ester per g dry weight sample applying the internal standard method using 20:2ω6 as a standard.

## Results

### Microparticulate diets

It appears from Table II that the ω3-HUFA content in SPB *Artemia* nauplii is considerably increased when the nauplii are fed during 24 h with a ω3-HUFA rich diet (*e.g.* CLORB and AA18). Only a slight increase is noticed when a ω3-HUFA lacking diet (RORB) is used for enrichment or when the nauplii are starved for 24 h. This suggests that *Artemia* nauplii are able to elongate the chain and desaturate lower fatty acids, be it not very efficiently. This bioconversion

Table II

ω3-HUFA content (area percent and mg fatty acid per g dry weight) of enrichment diets and *Artemia* preparations.
(-) not detected ; (tr) trace ; (Σω3-HUFA) sum ω3-HUFA > 20:3ω3 ; (24 hE) 24 h enriched ; (AS) after separation of nauplii ; (NS) no separation

| | 20:5ω3 | | 22:6ω3 | | Σω3-HUFA | |
|---|---|---|---|---|---|---|
| | Area % | mg/g | Area % | mg/g | Area % | mg/g |
| Enrichment diets | | | | | | |
| • CLORB | 8.0 | 6.3 | 10.0 | 5.2 | 20.9 | 14.2 |
| • RORB | – | – | – | – | tr | tr |
| • AA18 | 8.5 | 6.4 | 9.9 | 7.5 | 20.8 | 15.9 |
| • Selco | 11.2 | 78.2 | 13.2 | 91.8 | 30.4 | 212.6 |
| • Coconut | – | – | – | – | – | – |
| *Artemia* preparations | | | | | | |
| • SFB – freshly-hatched | 9.3 | 11.8 | 0.2 | 0.3 | 11.5 | 14.6 |
| • SPB – freshly-hatched | 0.5 | 0.5 | – | – | 0.7 | 0.8 |
| • SPB – 24 h starved | 1.4 | 1.1 | 0.6 | 0.4 | 3.5 | 3.0 |
| • SPB – CLORB, 24 hE | 6.3 | 7.3 | 1.5 | 1.9 | 8.9 | 10.1 |
| • SPB – RORB, 24 hE | 0.9 | 0.8 | – | – | 1.9 | 1.9 |
| • SPB – AA18, 24 hE | 8.2 | 9.9 | 1.5 | 2.4 | 10.6 | 13.9 |
| • GSL – freshly-hatched | 0.3 | 0.5 | – | – | 1.2 | 1.9 |
| • GSL – AS, 0.6 g coconut, 24 hE | 0.9 | 0.8 | 0.4 | 0.4 | 3.4 | 3.0 |
| • GSL – AS, 0.6 g Selco, 12 hE | 5.2 | 7.9 | 2.9 | 4.4 | 9.8 | 14.4 |
| • GSL – AS, 0.6 g Selco, 24 hE | 9.9 | 21.3 | 5.9 | 12.7 | 17.8 | 37.4 |
| • GSL – AS, 0.6 g Selco, 48 hE | 13.5 | 35.2 | 7.0 | 18.1 | 23.0 | 58.6 |
| • GSL – AS, 2 × 0.3 g Selco, 24 hE | 11.7 | 25.8 | 4.6 | 10.2 | 18.6 | 37.7 |
| • GSL – AS, 3 × 0.2 g Selco, 48 hE | 12.2 | 33.8 | 4.8 | 13.2 | 19.9 | 53.5 |
| • GSL – NS, 0.6 g Selco, 12 hE | 4.5 | 6.4 | 2.4 | 3.3 | 8.2 | 11.2 |
| • GSL – NS, 2 × 0.3 g Selco, 24 hE | 7.0 | 12.1 | 4.4 | 7.5 | 13.3 | 22.1 |
| • GSL – NS, 3 × 0.2 g Selco, 48 hE | 12.0 | 22.3 | 6.4 | 11.9 | 21.0 | 38.3 |
| • SUPAR | 4.6 | 8.0 | 4.0 | 6.8 | 9.7 | 16.7 |
| • TTS * | 13.0 | 13.6 | – | – | 15.6 | 16.3 |
| • TTS, decaps., NS, 2 × 0.3 g Selco, 19 hE * | 12.3 | 17.9 | 3.6 | 5.2 | 20.9 | 30.3 |
| • TTS, decaps., NS, 2 × 0.3 g Selco, 24 hE * | 12.6 | 26.8 | 4.1 | 8.9 | 20.5 | 45.2 |

* Data from Lisac *et al.* (1986).

ability was shown before in ongrown *Artemia* (Kayama *et al.*, 1963 ; Jezyck and Penicnak, 1966 ; Hinchcliffe and Riley, 1972).

The effect of ω3-HUFA enrichment on the nutritional value of *Artemia* nauplii is clearly illustrated in the culture test with *Mysidopsis bahia* (Table III) : a significant improvement in both survival and growth is obtained when ω3-HUFA-fortified nauplii were fed. Survival and growth (in terms of individual length) in *M. bahia* fed AA18-enriched *Artemia* is not significantly different from the treatment fed SFB nauplii.

EMULSIFIED DIETS

Table II and Fig. 1, 2, and 3 produce results of ω3-HUFA levels in GSL *Artemia* nauplii submitted to different enrichment techniques using the prepared emulsion Selco. When adding only one fraction of 0.6 g Selco/l, the ω3-HUFA concentration in separated nauplii increases from 1.9 mg/g after hatching to 14.4 mg/g after 12 h enrichment, to 37.4 mg/g after 24 h, and to 58.6 mg/g after 48 h enrichment. Splitting up 0.6 g Selco in two or three fractions does not yield increased levels. When enrichment is done without separating the nauplii from the hatching debris, *i.e.* by adding the emulsion directly to the hatching medium, a rationing is necessary for a 24 h and 48 h enrichment. One ration of 0.6 g Selco/l during 12 h enrichment gives similar ω3-HUFA levels as when separating the nauplii. However, the same application for a 24 h or 48 h enrichment resulted in a high naupliar mortality. Even when rationing the Selco in two or three fractions, the ω3-HUFA accumulation is always lower than enrichments with separated nauplii.

Enrichment during hatching incubation (Supar formulation) yields a significant increase in ω3-HUFA after about 30 h incubation (which coincides with about 6 h enrichment after the first Selco addition in previous procedures). The levels obtained after 36 h incubation are slightly higher than those after 12 h enrichment in the previous procedures. Survival of the nauplii, stocked at 300 individuals/ml was always above 90 % provided that the oxygen levels were maintained above 4 mg/l. In the procedure adding one portion of emulsion in the hatching medium for 24 h and 48 h enrichment oxygen depletion was the cause of high mortality.

When using separated nauplii and applying one or more rations of emulsion, no differences in survival nor enrichment success were noticed. Nonetheless for large scale application which involves more difficulties to maintain high oxygen levels, it is advisable to apply the emulsion in two fractions. Enrichment in the hatching medium requires disinfection of the cysts prior to incubation as to suppress bacterial blooming and oxygen demand. Good enrichment results have also been obtained in the hatching medium when using decapsulated cysts (Table II, Chinese cysts). Freshly-decapsulated cysts were incubated at 28 °C in 1 500 l seawater ; at maximal hatch (48 h) 0.3 g Selco/l was added and a second ration of 0.3 g/l was administered about 12 h later ; a sample of enriched *Artemia* was taken after 19 h and 24 h enrichment. Results show good ω3-HUFA accumulation when using this method.

The experiment on temperature and light effects on enrichment reveals that only temperature interacts (Table IV), *i.e.* ω3-HUFA buildup is significantly faster at 30 °C than at 25 °C. The effect of GSL *Artemia* enrichment with emulsified diets on their food value for mysid larvae is documented in Table V. Both survival and growth are significantly improved when feeding a ω3-HUFA rich diet. *Artemia* enrichment with a coconut oil emulsion does not improve survival in mysids although a significant effect on growth is noticed. This could be due to the higher

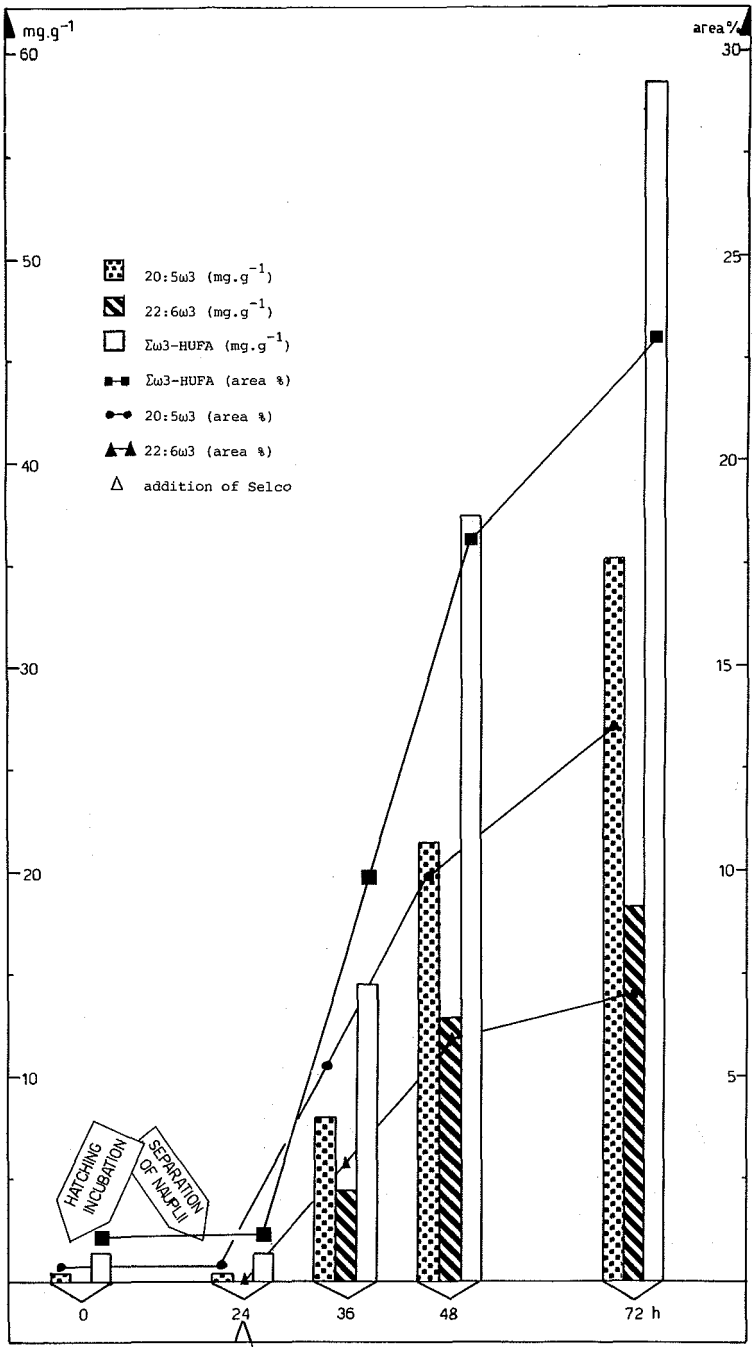


FIG. 1. Histographic presentation of ω3-HUFA accumulation (area % and mg/g values) in GSL *Artemia* nauplii enriched with a prepared emulsion (Selco), after separation from their hatching debris.

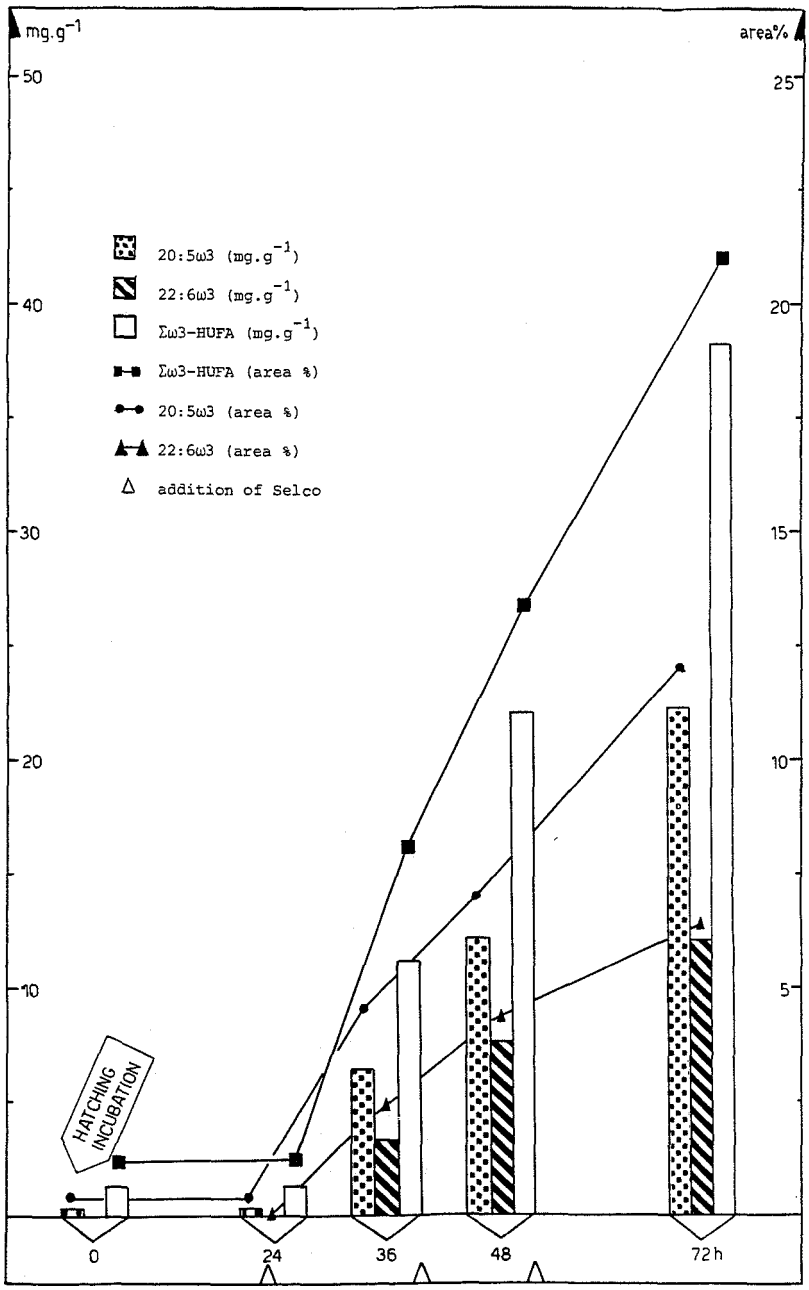


FIG. 2. Histographic presentation of ω3-HUFA accumulation (area % and mg/g values) in GSL *Artemia* nauplii enriched with a prepared emulsion (Selco) in the hatching medium.

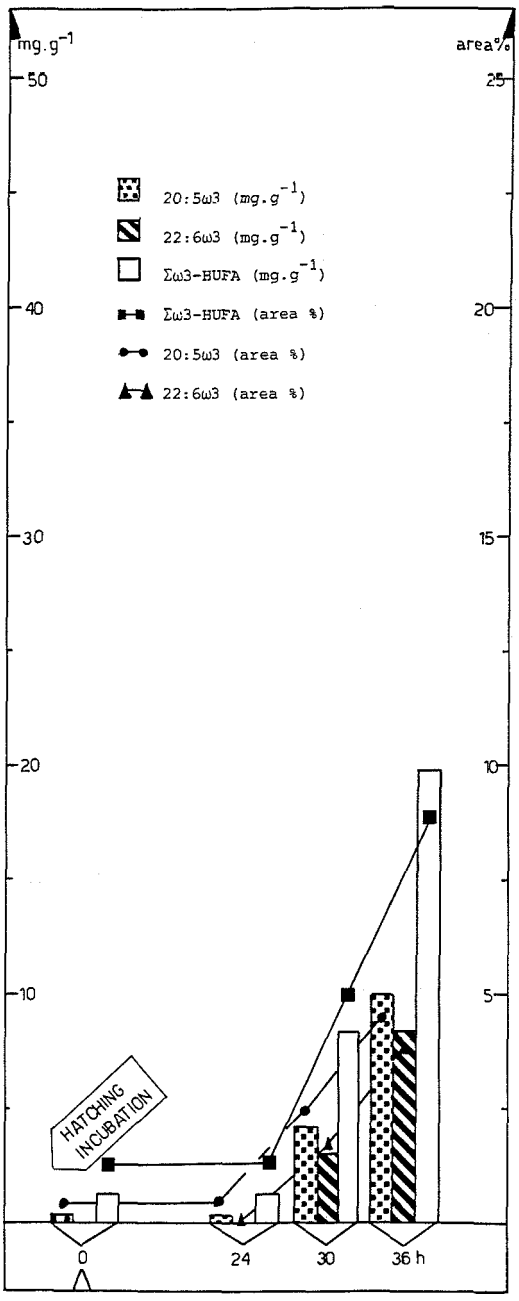


FIG. 3. Histographic presentation of ω3-HUFA accumulation (area % and mg/g values) in *Artemia* nauplii produced from treated cysts (Supar formulation), during hatching incubation.

TABLE III

Results of the *Mysidopsis bahia* culture test using *Artemia* nauplii freshly-hatched or enriched with microparticulate diets. (legend to symbols : see text)

| Culture results | SFB | SPB | | | | |
|---|---|---|---|---|---|---|
| | Newly hatched | Newly hatched | Starved 24 h | RORB 24 hE | CLORB 24 hE | AA18 24 hE |
| Survival (% ± sd) | $93.3 \pm 15.0^{aA}$ | $62.0 \pm 15.3^{cC}$ | $55.0 \pm 7.5^{cC}$ | $60.0 \pm 4.8^{cC}$ | $75.0 \pm 8.9^{bBC}$ | $92.5 \pm 13.1^{aAB}$ |
| Individual length (µm ± sd) | $5532 \pm 471^{aA}$ | $4587 \pm 270^{bBC}$ | $4504 \pm 214^{bBC}$ | $4285 \pm 135^{bC}$ | $5029 \pm 284^{aAB}$ | $5375 \pm 415^{aA}$ |
| Individual dry weight (µg ± sd) | $354 \pm 47^{aA}$ | $198 \pm 30^{cBC}$ | $216 \pm 28^{bcBC}$ | $188 \pm 16^{cC}$ | $259 \pm 29^{bB}$ | $259 \pm 33^{bB}$ |

a,b,c resp. A,B,C : means with a different superscript are significantly different at the $\alpha$ : 0.05 resp. $\alpha$ : 0.01 level.

TABLE IV

ω3-HUFA accumulation in separated GSL *Artemia* nauplii under different conditions of light and temperature using a prepared emulsion (Selco) during 24 h

| | 20:5ω3 | | 22:6ω3 | | Σω3-HUFA | |
|---|---|---|---|---|---|---|
| | Area % | mg/g | Area % | mg/g | Area % | mg/g |
| Light, 25 °C | $7.4 \pm 0.2^{b}$ | $10.9 \pm 0.7^{b}$ | $4.0 \pm 0.7^{a}$ | $5.9 \pm 1.1^{b}$ | $16.3 \pm 0.3^{ab}$ | $23.9 \pm 0.9^{b}$ |
| Light, 30 °C | $8.7 \pm 0.4^{a}$ | $15.5 \pm 0.6^{a}$ | $4.3 \pm 0.2^{a}$ | $7.7 \pm 0.3^{a}$ | $18.7 \pm 0.1^{a}$ | $33.3 \pm 0.1^{a}$ |
| Dark, 25 °C | $7.5 \pm 0.8^{ab}$ | $11.7 \pm 1.6^{b}$ | $3.9 \pm 0.7^{a}$ | $6.0 \pm 1.3^{b}$ | $15.4 \pm 1.7^{b}$ | $23.9 \pm 3.1^{b}$ |

a,b : means with a different superscript are significantly different at the $\alpha$ : 0.05 level.

energetic content of the nauplii after lipid enrichment and/or to the slightly increased level of 22:6ω3. This effect does not appear with SPB *Artemia* as a food source where starved nauplii also contain low but notable levels of 22:6ω3.

TABLE V

Results of the *Mysidopsis bahia* culture test using GSL *Artemia* nauplii freshly hatched or enriched with an emulsion (Selco or coconut oil)

| Culture results | Freshly-hatched | Coconut oil 24hE | Selco 24hE |
|---|---|---|---|
| Survival (% ± sd) | $45.8 \pm 27.5^{b}$ | $45.6 \pm 12.8^{b}$ | $78.1 \pm 13.5^{a}$ |
| Individual length (μm ± sd) | $4\,087 \pm 200^{cB}$ | $4\,482 \pm 337^{bB}$ | $5\,108 \pm 135^{aA}$ |
| Individual dry weight (μg ± sd) | $205.4 \pm 20.0^{bB}$ | $256.8 \pm 47.4^{bB}$ | $325.3 \pm 14^{aA}$ |

a,b,c resp. A,B,C : means with different superscript are significantly different at the α :0.05 resp. α : 0.01 level.

## Discussion and conclusions

Aiming to enhance the nutritional value of *Artemia* nauplii ω3-HUFA enrichment may be done in various ways using microparticulate or emulsified diets. The present study reveals that the highest levels are obtained when using the prepared emulsion. These levels are notably higher than those reported earlier in literature (review in Léger *et al.*, 1986). This may be due to the optimized procedures used for *Artemia* hatching and enrichment, and also to the high stability of the emulsion in strongly aerated seawater. The availability in the water column of a sufficiently high concentration of enrichment particules or droplets of an appropriate size is indeed critical for optimal enrichment in *Artemia* (cf. better enrichment levels when using AA18 – with better granulometric composition – versus CLORB, although both diets contain similar ω3-HUFA concentrations ; higher levels in Selco-enriched nauplii as compared to literature results with other emulsions). Contrary to the low naupliar densities reported by other authors (*e.g.* 10-15/ml, Robin, 1982 ; Robin *et al.*, 1984) we still obtain higher enrichment levels with up to 300 nauplii/ml. Application of optimal hatching procedures, reaching maximal hatch in a minimal time, further helps in reaching high ω3-HUFA levels after only short enrichment periods. Attention has also to be paid as to have the enrichment diet available at the time of first feeding of the nauplii (instar II-stage). This time is determined by the hatching rate of the cysts used (Vanhaecke and Sorgeloos, 1982). Another intrinsic factor determining maximal enrichment rates is the hatching synchrony of the *Artemia* cysts used (*e.g.* time lapse between appearance of first and last hatching nauplius) which can vary from 5 h to 17 h at 25 °C (Vanhaecke, 1983). This means that the moment of first feeding (= start of enrichment) will also be spread. Taking these hatching characteristics into account, higher enrichment levels can be achieved in shorter times, and as such smaller sized *Artemia* metanauplii are yielded. Indeed, 12 h to 48 h enriched *Artemia* metanauplii, produced according to the procedures described in this study measure 660 μm (12 h enrichment) to 790 μm (48 h enrichment) instead of over 900 μm when other methods are applied. The literature provides numerous examples of prey size being an important parameter in larval culture success, since the larger it is, (*e.g.* enriched

*Artemia*) the more delayed its earliest introduction in the feeding regime (Léger *et al.*, 1986) will be.

The culture test with *Mysidopsis bahia* illustrates the beneficial effect on survival and growth of feeding ω3-HUFA enriched *Artemia*. When feeding enriched SPB *Artemia*, survival and growth (in terms of length) of *M. bahia* are not significantly different from the treatment fed SFB nauplii. Growth in terms of individual dry weight, however, is best in mysids fed SFB *Artemia*. This might confirm that SPB *Artemia* are not only deficient in ω3-HUFA but that they may contain particular antinutrients as suggested by Olney *et al.* (1980). These observations could also indicate that, for *M. bahia*, the sum of ω3-HUFA is more important than the contribution of particular fatty acids, *e.g.* 22:6ω3. On the other hand the effect of ω3-HUFA enrichment in GSL *Artemia* as a food source for *M. bahia* is very clear on both survival and growth. When growth in terms of individual dry weight is compared with the SFB-treatment (previous test), we see that the ω3-HUFA enriched GSL *Artemia* are still inferior. This is, however, not the case with other predators such as shrimp larvae *Penaeus stylirostris* (Léger *et al.*, 1985) and *Penaeus vannamei* (Léger *et al.*, 1987), and fish larvae *Dicentrarchus labrax* (Franičević *et al.*, 1987) and *Sparus aurata* (Lisac *et al.*, 1986) where SPB, GSL, or other *Artemia* nauplii enriched with the same ω3-HUFA diet always performed significantly better than a SFB-control. For these predators the presence of the 22:6ω3 fatty acid in enriched *Artemia* was believed to be an essential factor, confirming the role of 22:6ω3 as an essential component in the larval nutrition of marine fish and crustaceans (Yone, 1978 ; Holland and Jones, 1981 ; Léger and Frémont, 1981 ; Léger *et al.*, 1985 ; Bell *et al.*, 1985). Since this essential fatty acid is mostly absent in *Artemia* and the 20:5ω3 content varies considerably it is advised to apply ω3-HUFA enrichment techniques as a routine procedure in the larval culture of marine fish and shrimp species. The enrichment techniques described in this paper may also be applied for incorporating other essential nutrients (*e.g.* vitamines, amino acids, etc.) and other active components (pigments, therapeutics, etc.) in *Artemia*. This will be presented in other studies.

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## Literature cited

BELL M. V., R. J. HENDERSON, B. J. S. PIRIE, and J. R. SARGENT. 1985. Effects of dietary polyunsaturated fatty acid deficiencies on mortality, growth and gill structure in the turbot, *Scophthalmus maximus*. J. Fish Biol. 26:181-191.

BLIGH E. G. and W. J. DYER. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37:911-917.

BROMLEY P. J. 1978. The weaning of hatchery reared turbot larvae (*Scophthalmus maximus*) on a dry diet. Aquaculture 13:339-345.

ENRIGHT C. T. 1984. The growth response of juvenile European cysters fed selected and biochemically manipulated phytoplankton species. Paper presented at 15th Annu. Meet. World Maricult. Soc. Vancouver, British Columbia, March 18-22, 1984.

FORSTER J. R. M. and J. F. WICKINS. 1967. Experiments on the culture of the prawn Palaemon serratus. ICES, Maricult. Comm. E : 13. 9 p.

FRANIČEVIĆ V., D. LISAC, J. BUBLE, Ph. LÉGER, and P. SORGELOOS. 1987. International Study on *Artemia.* XLII. The effect of the nutritional quality of *Artemia* on the growth and survival of seabass (*Dicentrarchus labrax* L.) larvae in a commercial hatchery. p. 139-145. In : Production in marine hatcheries. Loix B. (Ed.). MEDRAP-FAO. Salammbo, Tunesie. 387 p.

GATESOUPE J. 1982. Nutritional and antibacterial treatments of live food organisms : the influence on survival, growth rate and weaning success of turbot (*Scophthalmus m.*). Ann. Zootech. (Paris) 31:353-368.

HINCHCLIFFE P. R. and J. P. RILEY. 1972. The effect of diet on the component fatty acid composition of *Artemia salina.* J. Mar. Biol. Ass. U.K. 52:203-211.

HOLLAND P. L. and D. A. JONES. 1981. Microencapsulated feed enhances the dietary value of *Artemia.* Fish Farm. Int., Dec. 1981. p. 17.

HOWELL B. R. 1979. Experiments in the rearing of larval turbot (*Scophthalmus maximus*). Aquaculture 18:215-225.

HOWELL B. R., P. J. BROMLEY, and T. C. ADKINS. 1981. The food value of *Artemia* for larval turbot (*Scophthalmus maximus*) : effects on survival, growth and acceptance of a compound feed. ICES, Maricult. Comm. F : 10.

JEZYCK P. F. and A. J. PENICNAK. 1966. Fatty acid relationship in an aquatic food chain. Lipids 1 (6):427-429.

JONES P. A., D. L. HOLLAND, and S. S. JABORIE. Current status of microencapsulated diets for aquaculture. J. Appl. Biochem. Biotechn. (in press).

KAYAMA M., X. TSUCHIYA, and J. F. MEAD. 1963. A model experiment of aquatic food chain with special significance of fatty acid conversion. Bull. Jap. Soc. Sci. Fish. 29(5):452-458.

KELLY R. O., A. W. HASELTINE, and E. E. EBERT. 1977. Mariculture potential of the spot prawn, *Pandalus platyceros* Brandt. Aquaculture 10:1-16.

LAVENS P., Ph. LÉGER, and P. SORGELOOS. 1987. Manipulation of the fatty acid profile in *Artemia* offspring produced in intensive culture systems. Paper presented at the Aquaculture Europe 1987 Int. Conf., RAI, Amsterdam (The Netherlands), June 2-5, 1987.

LÉGER C. and L. FRÉMONT. 1981. Métabolisme des acides gras et des lipides neutres. p. 215-246. In : Nutrition des poissons. CNRS (Ed.). Paris.

LÉGER Ph., D. A. BENGTSON, K. L. SIMPSON, and P. SORGELOOS. 1986. The use and nutritional value of *Artemia* as a food source. Oceanogr. Mar. Biol. Annu. Rev. 24:521-523.

LÉGER Ph., D. A. BENGTSON, P. SORGELOOS, K. L. SIMPSON, and A. D. BECK. 1987. The nutritional value of *Artemia* : a review. In : *Artemia* research and its applications. Vol. 3. Sorgeloos P., D. A. Bengtson, W. Decleir, and E. Jaspers (Eds). Universa Press, Wetteren, Belgium.

LÉGER Ph., G. F. BIEBER, and P. SORGELOOS. 1985. International Study on *Artemia.* XXXIII. Promising results in larval rearing of *Penaeus stylirostris* using a prepared diet as algal substitute and for *Artemia* enrichment. J. World Maricult. Soc. 16:354-367.

LÉGER Ph., R. CHAMORRO, and P. SORGELOOS. 1987. Improved hatchery production of postlarval *Penaeus vannamei* through application of innovative feeding strategies with an algae substitute and enriched *Artemia.* Paper presented at World Maricult. Soc. 18th Annu. Meet., Guayaquil (Ecuador), Jan. 18-23, 1987.

LÉGER Ph., M. D. JOHNS, and P. SORGELOOS. 1987. Description of a standard bioassay test with the marine crustacean *Mysidopsis bahia* (*M.*) for the evaluation of the nutritional effectiveness of *Artemia* nauplii and metanauplii. In : *Artemia* research and its applications. Vol. 3. Sorgeloos P., D. A. Bengtson, W. Decleir, and E. Jaspers (Eds). Universa Press, Wetteren, Belgium.

LÉGER Ph., P. SORGELOOS, O. MILLAMENA, and K. L. SIMPSON. 1985. International Study on *Artemia.* XXV. Factors determining the nutritional effectiveness of *Artemia* : the relative impact of chlorinated hydrocarbons and essential fatty acids in San Francisco J. Exp. Mar. Biol. Ecol. 93:71-82.

LISAC D., V. FRANIČEVIĆ, Z. VEJMELKA, J. BUBLE, Ph. LÉGER, and P. SORGELOOS . 1986. International Study on *Artemia.* XLIII. The effect of live food fatty acid content on growth and survival of seabream (*Sparus aurata*) larvae. Paper presented at the Conf. Ichthyopathology in Aquaculture, Oct. 21-24, 1986, Inter Univ. Cent., Dubrovnik, Yugoslavia.

MOAL J., J. F. SAMAIN, and J. R. LE GOZ. 1978. C/N et contrôle de la physiologie des cultures de phytoplancton. p. 141-148. In : Physiology and behaviour of marine organisms, Proc. 12th Europe. Mar. Biol. Symp. Mc Lusky D. S. and A. J. Berry (Eds). Performance Press, Oxford and New York.

MORRIS R. W. 1956. Some aspects of the problem of rearing marine fishes. Bull. Mus. Oceanogr. 1 082. 62 p.

OLNEY C. E., P. S. SCHAUER, S. McLEAN, T. LU, and K. L. SIMPSON. 1980. International Study on *Artemia*. VIII. Comparison of the chlorinated hydrocarbons and heavy metals in five different strains of newly hatched *Artemia* and a laboratory reared fish p. 343-352. In : The brine shrimp *Artemia*. Vol. 3. Ecology, Culturing, Use in aquaculture, Persoone G., P. Sorgeloos, O. Roels, and E. Jaspers (Eds). Universa Press, Wetteren, Belgium. 428 p.

ROBIN J. H. 1982. Comparaison de deux méthodes d'amélioration de la valeur alimentaire d'*Artemia salina* pour nourrir les larves de bar (*Dicentrarchus labrax*). ICES Maricult. Comm. F:13. 11 p.

ROBIN J. H., F. J. GATESOUPE, and R. RICARDEZ. 1981. Production of brine shrimp (*Artemia salina*) using mixed diets : consequences on rearing of seabass larvae (*Dicentrarchus l.*). J. World Maricult. Soc. 12(2):119-120.

ROBIN J. H., F. J. GATESOUPE, G. STÉPHAN, H. LE DELLIOU, and G. SOLAUN. 1984. Méthodes de production de filtreurs-proies et amélioration de leur qualité alimentaire. Oceanis 10(5):497-504.

SCHAUER P. S. and K. L. SIMPSON. 1978. Effects of diets on the fatty acid composition of juvenile Atlantic silverside (*Menidia menidia*). p. 175-187. In : Prov. 9th Annu. Meet. World Maricult. Soc. Avault I. W., Jr. (Ed.). Louisiana State Univ., Baton Rouge, 807 p.

SCOTT J. M. and C. MIDDLETON. 1979. Unicellular algae as a food for turbot (*Scophthalmus maximus L.*) larvae – the importance of dietary long chain polyunsaturated fatty acids. Aquaculture 18:227-240.

SORGELOOS P., E. BOSSUYT, P. LAVENS, Ph. LÉGER, P. VANHAECKE, and D. VERSICHELE. 1983. The use of brine shrimp *Artemia* in crustacean hatcheries and nurseries. p. 71-96. In : CRC Handbook of mariculture. Vol. 1. Crustacean aquaculture. McVey J. P. (Ed.). CRC Press, Inc., Boca Raton, Florida, USA, 442 p.

VANHAECKE P. 1983. Vergelijkende studie van diverse geografische rassen van het pekelkreeftje, *Artemia*, ter verbetering van zijn gebruik in de aquacultuur. Thesis, State Univ. Ghent, Fac. Agr. Sc., Gent, Belgium. 420 p.

VANHAECKE P. and P. SORGELOOS. 1982. International study on *Artemia*. XVIII. The hatching rate of *Artemia* cysts- a comparative study. Aquacult. Eng. 1 : 263-273.

WATANABE T., F. OOWA, L. KITAJIMA, and S. FUJITA. 1978. Nutritional quality of *Artemia salina* as a living feed from the viewpoint of essential fatty acids for fish. Bull. Jap. Soc. Sci. Fish. 44(10):1115-1112.

WATANABE T., F. OOWA, C. KITAJIMA, and S. FUJITA. 1980. Relationship between dietary value of brine shrimp *Artemia salina* and their content of ω3-HUFA. Bull. Jap. Soc. Sci. Fish. 46(1):35-41.

WATANABE T., M. OHTA, C. KITAJIMA, and S. FUJITA. 1982. Improvement of the dietary value of brine shrimp *Artemia salina* for fish by feeding them on ω3 highly unsaturated fatty acids. Bull. Jap. Soc. Sci. Fish. 48:1775-1782.

WATANABE T., T. TAMIYA, A. OKA, M. HIRATA, C. KITAJIMA, and S. FUJITA. 1983. Improvement of the dietary value of live foods for fish larvae by feeding them on ω3 highly unsaturated fatty acids and fat soluble vitamins. Bull. Jap. Soc. Sci. Fish. 49(3):471-479.

WICKINS J. F. 1976. Prawn biology and culture. Oceanogr. Mar. Biol. Annu. Rev. 14:435-507.

YONE Y. 1978. Essential fatty acids and lipid requirements of marine fish. p. 43-59. In : Dietary lipids in aquaculture. Jap. Soc. Sci. Fish. (Ed.). Suisangaku Ser. no. 22, Koseisha Koseikaku, Tokyo.