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Use of Rotifers Cultured on Different Microalgal Species in Larval Feeding of Sea Bass *Dicentrarchus labrax*

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Abstract

A larval feeding trial was carried out to study the use of rotifers cultured on different algal species in larval feeding of sea bass (*Dicentrarchus labrax*, Serranidae) from 0 to 24 days post hatch, and to study their effects on the survival rate and growth of larvae. Three different populations of the rotifers *Brachionus plicatilis* Müller were cultured on different algal species, *Tetraselmis chuii* (diet A), *Chlorella vulgaris* (diet C), and a mixture of both at a ratio of 1:1 by volume (diet B) as a long time enrichment with omega three-highly unsaturated fatty acids (n_3–HUFA). Rotifers were added to the larvae from the 3rd to the 17th day to keep 3 to 10 indi. ml^{-1} of rearing water. However feeding with *Artemia* was started on the 10th day in all treatments.

Results indicated that eicosapentaenoic acid (EPA 20:5n_3) content was high in *Tetraselmis* and very low in *Chlorella*. EPA contents were 5.11, 2.31 and 0.58% of total lipid for rotifers fed on diets A, B and C, respectively. However, docosahexaenoic acid (DHA, 22:6n_3) was absent in either rotifers or algae. Survival rate was consistently increased when larvae were fed on diet A (included rotifers fed on *Tetraselmis* as alone) followed by those fed on diet B (included rotifers fed on mixture of *Tetraselmis* and *Chlorella*). The poorest survival rate was obtained when larvae were fed on diet C (included rotifers fed on C*hlorella* alone). Survival rate values were 21.20, 19.49 and 13.02 % for *D. labrax* larvae fed on diets A, B and C, respectively. To sum up, survival rate and growth of sea bass larvae were improved as EPA content increased in their feed (algae or rotifers). The same trend was found for final length, gain and growth indices.

Introduction

Production and rearing of fish larvae have been identified as a major constraint to many aquaculture processes (Sukenik et al. 1993). The availability of a reliable and highly nutritional larval food is one of the crucial demands at this production stage. Rotifer *Brachionus plicatilis Rotifera* has been widely used in marine hatcheries due to its ideal size, fast reproductive rate and its ability to feed on different unicellular algae (Witt et al. 1984), formulated diets (Lavens et al. 1994) and baker's yeast. However, rotifers fed on diets and yeast are transferred to high algal concentrations for a short time to improve their nutritional quality prior to use as feed for fish larvae (Watanabe et al. 1983; Koven et al. 1989; Volkman et al. 1989). The chemical composition of rotifers is similar to that of the algae upon which they fed (Ben-Amotz et al. 1987; Caric et al. 1993).

Therefore, enrichment of rotifer with n_3-HUFA by feeding them with rich sources of n_3 can be accomplished through feeding on different microalgae (Watanabe et al. 1979; Watanabe et al. 1983; Koven et al. 1989). The majority of the marine fish larvae required unsaturated fatty acids such as EPA, 20:5n_3 and DHA, 22:6n_3. The high levels of EPA may have affected pigmentation (Rainuzzo et al. 1997). Kanazawa et al. (1982) reported that EPA was preferentially incorporated into the gall bladder, swim bladder and alimentary tract, and secondly into the liver and gill tissue. The high levels of EPA may have affected pigmentation (Rainuzzo et al. 1997). In addition, EPA is the major fatty acid present in lipids of marine fish eggs such as red sea bream *Pagrus major* (Izquierdo et al. 1989) and gilthead sea bream, *Sparus aurata* (Mourente and Odriozola 1990).

Addition of microalgae to rotifers as a long term enrichment technique (combined growth and n_3 HUFA enrichment during the production phase of rotifers) seems to be very efficient in obtaining a high n_3 HUFA content in rotifers while maintaining a normal lipid content (Rainuzzo et al. 1997). Considerable differences have been noted in the survival rate of larvae fed on rotifers reared on diverse algae cultures (Dendrinos and Thorpe 1987; Rainuzzo et al. 1997).

The aim of this study was to investigate the effect of dietary eicosapentaenoic acid (EPA, 20:5n_3) levels, supplied in the rotifers cultured on different microalgae (*Chlorella vulgaris*, *Tetraselmis chuii* and a mixture of both), on survival and growth of European sea bass *D. labrax* larvae 24 days after hatching.

Materials and Methods

Sea bass larvae

Larvae used in this study were obtained from the Maturation and Spawning Division of the Hatchery of Mariculture Research Center (MRC), Suez Canal University, El-Arish, Egypt. Broodstock of fish were collected

from the Bradawil Lagoon. They were transported in fiberglass tanks supplied with oxygen.Upon arrival at the hatchery, fish were acclimated and stocked in 20 m^3 conical-bottomed fiberglass tanks for maturation and spawning. Photoperiodical manipulation was used to induce ovarian maturation as described by Ahmad (1999). Spawned eggs were incubated in 125 l conical-bottomed fiberglass tanks. After hatching, larvae were carefully collected in the morning and immediately transferred to be stocked in nine 60 l glass aquariums, three aquariums per treatment. Each was stocked with 1500 larvae.

Rotifers B. plicatilis

Long time enrichment technique of rotifers was done by rearing three different populations of the rotifers *B. plicatilis* on different algal species *Tetraselmis chuii* (diet A), *Chlorella vulgaris* (diet C) and a mixture of both at a ratio of 1:1 by volume (diet B). Rotifers were cultured in eight transparent fiberglass tanks, each with 300 L-capacity. The rotifers were maintained at 20 ± 1°C, 20 ppt salinity and 800 lux light intensity in 12/12 hrs L/D daily photoperiod regime. Rotifers were harvested in a 40 µm mesh size plankton bucket and rinsed with clear seawater before being fed with experimental larvae.

Microalgae

The starter inoculations of test algal species were obtained from SEAFDEC, Iloilo, Philippines. Algae were cultured in seawater supplemented with culture medium of Walne (1966) and maintained in a chamber with controlled conditions of temperature 20 ± 2°C, 20 ppt salinity and illumination of 1000 lux for 12/12 hrs, L/D daily. Glass flasks of 0.5 to 2 l capacity were used for stock culture with an inoculation density of 5000 to 10000 cell·ml^{-1} as described by Lavens and Sorgeloos (1996). Peak density of 5 to 15 x 10^6 cell· ml^{-1} occurred within 4 to 5 days after inoculation. Once peak density was attained, the starter stock culture was used for subculture or mass culture immediately. Mass culture was conducted in large fiberglass tanks (2.0-m^3 capacity) and used to feed rotifers.

Artemia

Artemia cysts of the Great Salt Lake (GSL), Arganta, USA were treated with sodium hypochlorite for decapsulating. They were incubated in seawater to hatch as described by Lavens and Sorgeloos (1996).

Experimental conditions for larval rearing

Aquariums were provided with filtered seawater and fine aeration. Experiment was carried out under natural light. The water quality in the larval aquariums was maintained through partial change of water starting from the 2nd day onwards. About 30 to 100% of the water volume was replaced daily with new filtered seawater. Dissolved oxygen was maintained using an air

blower through air stones. The experimental larvae were reared at ambient salinity 35 ppt and temperature 16 ± 2°C. Experiment lasted for 24 days. Dead larvae were counted and removed daily. Rotifers were added to the larvae from the 3rd to the 17th day and kept at a density of 3 to 10 indi. ml^{-1} of rearing water. However feeding with *Artemia* was started on the 10th day. The schedule of larval feeding of sea bass used in this work is presented in table 1.

Analytical methods

Total lipids were extracted according to Folch et al. (1957) by homogenization of samples in chloroform:methanol mixture (2:1, v/v). Fatty acids were determined by using gas liquid chromatography (GLC) in methyl ester form as described by Nelson et al. (1969).

Total length was determined by measuring 20 larvae per aquaria weekly using an ocular micrometer and the number of larvae was counted.

Terminologies used:

1. Survival rate (%) = 100(final count/initial count)
2. Gain of larval length (mm) = final length –initial length.
3. Growth index (%/day) =100(Ln L_t –Ln L_o)/ days. Where L_t is the final length, L_o is the initial length and Ln is normal log.

Statistical analysis

Analysis of variance (ANOVA) was carried out according to Snedecor and Cochran (1982) using a complete randomized design (CRD). Differences were subjected to Duncan's Multiple Range test.

Results and Discussion

Table 2 shows the fatty acids profile of marine microalgae and rotifers used in the present study. The results showed that *Chlorella* had a higher percentage of 16:0, 18:1 n_9, 18:2 n_6, than those of *Tetraselmis*. However, *Tetraselmis* contained 16:1 n_3, 17:0, 18:3 n_3, 20:4 n_6, 20:5 n_3 more than those in *Chlorella*. Fatty acids of 15:0, 16:2 n_6, 20:2 n_6, 20:5 n_3 and 22:6 n_3 were absent in *Chlorella* but *Tetraselmis* didn't have acids of 15:0, 20:2 n_6, 20:3 n_6 and 22:6 n_3.

EPA was found with 5.11, 2.31 and 0.58% of total lipids in rotifers fed on *Tetraselmis* and mixture of *Tetraselmis* and *Chlorella* and *Chlorella,* respectively. In addition, DHA 22:6n_3 was absent in either rotifers or in their diets. Similar findings were recorded by Scott and Middleton (1979); Ben-Amotz et al. (1987); Whyte and Nagata (1990); Cariç et al. (1993). Whyte and Nagata (1990) reported that fatty acid profiles of rotifers and corresponding diets fed to the rotifers indicated transfer and storage of major fatty acid constituents in the feeding process. They also suggested an endogenous synthesis of these acids and/or assimilation and concentration of minor amounts that were not evident in the profiles of the diets.

Successful rearing of marine fish depends on a good adequacy between the specific dietary requirements and the biochemical composition of the fish prey. The level of n_3-HUFA in the prey is considered a major factor for dietary value particularly arachidonic acid (20:4 n_6), EPA and DHA. Rotifers fed on *Chlorella* were poor in these fatty acids. However rotifers fed on *Tetraselmis* had more EPA than those fed on *Chlorella* (Table 2). EPA content was found to be 5.11, 2.31 and 0.58% of total lipids in tissues of rotifers fed on *Tetraselmis*, mixture of *Chlorell*a and *Tetraselmi*s and *Chlorell*a, respectively. Both Rainuzzo et al. (1997) and Reintan et al. (1997) found improvement in the rotifers, *B. plicatilis* as feed for marine fish larvae has been achieved through enrichment with various diets containing different levels of n_3-HUFA by feeding with algae rich in essential fatty acids for a short time (24 hrs prior to feed for larvae) or a for a longer time (through culture period of rotifers).

Table 1. Schedule of larval feeding of experimental sea bass larvae.

Age of larvae (day after hatching)	Feeding source (indi. ml^{-1})	
	Rotifer	Artemia
0-2	0	-
3	3	-
4-5	5	-
6-9	10	-
10-12	10	0.5
13-15	10	1.0
16-17	5	1.5
18-20	-	1.5
21-24	-	3.0

Table 2. Fatty acids profile of rotifers and their feed of algae (% of total lipids).

Fatty acid	*Tetraselmis*		*Tetraselmis* + *Chlorella*		*Chlorella*	
	Algae	Rotifers	Algae	Rotifers	Algae	Rotifers
14:0	0.37	1.15	0.42	2.00	0.29	3.65
14:1n_3	0.31	0.71	0.35	0.81	0.38	0.60
15:0	-	0.25	-	0.11	-	0.49
16:0	13.8	13.51	26.9	15.21	43.6	20.8
16:1n_3	6.01	1.00	3.71	1.21	1.32	0.91
16:2n_6	0.39	0.43	0.23	0.32	-	0.18
17:0	2.31	1.56	1.10	1.36	0.37	-
18:0	0.22	3.77	5.01	8.07	0.69	2.98
18:1n_9	2.90	-	3.21	3.96	4.61	-
18:2n_6	10.08	7.79	14.11	10.88	20.1	13.32
18:3n_3	12.09	15.65	7.12	9.13	2.69	0.02
20:2n_6	-	0.41	-	0.59	-	0.79
20:3w$_6$	-	0.81	0.27	4.10	0.41	7.00
20:4w$_6$	1.11	3.11	0.59	5.09	0.25	4.97
20:5n_3	6.15	5.11	3.16	2.31	-	0.58
22:6n_3	-	-	-	-	-	-
Lipids* %	12.29	13.11	9.91	10.42	8.68	9.78

* % on the dry weight basis

In the present study, results showed that survival rate consistently increased when larvae were fed on diet A (included rotifers fed on *Tetraselmis* alone) followed by those fed on diet B (included rotifers fed on mixture of *Tetraselmis* and *Chlorella*). The poor survival rate of larvae was obtained when larvae were fed on diet C (included rotifers fed on C*hlorella* alone). Survival rates were 21.20, 19.49 and 13.02 for diets A, B and C, respectively (Table 3). The same trend was found for final length, gain and growth index (Tables 4 and 5). The same results were obtained by Samocha (1984); Smith et al. (1986); and Telley et al. (1988). For example, El-Dakar (1998) used five species of microalgae including *Tetraselmis* and *Chlorella* as alone or mixed with other algal species in shrimp larval feeding. He indicated that higher survival rate, metamorphosis and growth index were observed clearly when larvae were fed on *Tetraselmis*. However larvae fed on *Chlorella* gave poor results. Survival rate values were 79.7 and 27.0% for larvae fed on *Tetraselmis* and *Chlorella*, respectively. In addition, marine fish larvae have been reported to be unable to synthesize *de novo* polyunsaturated fatty acids of either n_6 or n_3 (Kanazawa et al.1979). On the other hand, rotifers have been found to synthesize only minor amounts of n_3 polyunsaturated fatty acids (Lubens et al. 1985). Therefore, these acids must be provided by the rotifer diet to meet the requirements of fish larvae. Results of Toner (1975); Rodde et al.(1976); and Epifanio et al.(1976) showed that far better growth is obtained with mixtures of two or more species of algae than with either mono-species.

Table 3. Survival rate (%)* of sea bass *D. labrax* larvae fed rotifer cultured on different microalgae (mean ± SD).

Days**	Feed source of rotifers		
	Tetraselmis (A)	Tetraselmis + Chlorella (B)	Chlorella (C)
0	100.0±0.05^{a}	100.00±0.00^{a}	100.00±0.00^{a}
9	77.780±0.35^{ab}	78.670±0.20^{a}	76.890±0.24^{b}
14	58.500±0.35^{a}	51.10 ±0.51^{b}	46.990±0.31^{c}
19	30.290±0.44^{a}	27.600±0.47^{b}	18.130±0.41^{c}
24	21.200±0.55^{a}	19.490±0.75^{b}	13.020±0.50 ^{c}

*Values in the row having a common superscript letter are not significantly different (P> 0.05).
** day after hatching.

Table 4. Total length* of sea bass *D. labrax* larvae fed rotifer cultured on different micro algae (mean ± SD).

Days**	Feed source of rotifers		
	Tetraselmis (A)	Tetraselmis + Chlorella (B)	Chlorella (C)
0	3.50±0.15^{a}	3.50± 0.02^{a}	3.50±0.01 ^{a}
9	4.21±0.06^{a}	4.30±0.08^{a}	3.85±0.04^{b}
14	5.40±0.13^{a}	5.35±0.15^{a}	5.00±0.06^{b}
19	6.70±0.27^{a}	6.51±0.20^{ab}	6.30±0.16^{b}
24	7.92±0.30^{a}	7.64±0.31^{a}	7.01±0.26^{b}

*Values in the row having a common superscript letter are not significantly different (P>0.05).
** day after hatching.

The present study used three treatments to achieve 5.11, 2.31 and 0.58% in total lipid of EPA, the corresponding diet A (rotifers fed on *Tetraselmis*), diet B (mixture of *Chlorell*a and *Tetraselmi*s species) and diet C (rotifers fed on *Chlorella*). Results indicated that survival and growth of *D. labrax* larvae were

Table 5. Growth performance* of sea bass *D. labrax* larvae fed rotifer cultured on different microalgae (mean ± SD).

Feed sources	Initial size (mm)	Final size (mm)	Gain (mm)	Gain relative (%)	Growth index (%/day)
Tetraselmis	3.50±0.0[a]	7.92± 0.30[a]	4.42±0.10[a]	125.7±2.7[a]	3.40±0.06 [a]
Tetraselmis+ Chlorella	3.50±0.0[a]	7.64±0.31[b]	4.13±0.6[b]	118.0±1.8[b]	3.25±0.04[b]
Chlorella	3.50±0.0[a]	7.01±0.25[c]	3.51±0.09[c]	100.0±2.7[c]	2.89±0.07[c]

*Values in the column having a common superscript letter are not significantly different (P>0.05).

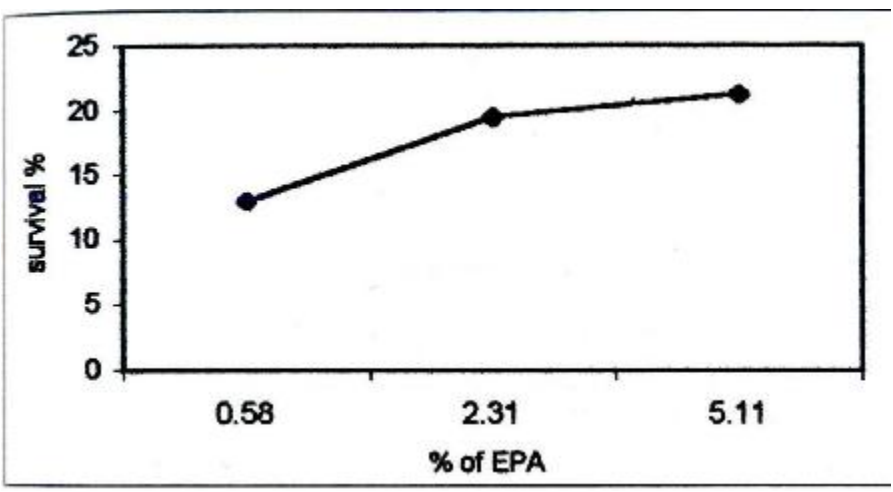

Fig. 1. Effect of different dietary levels of EPA on survival rate of sea bass *D. labrax* larvae after 24 days from hatching.

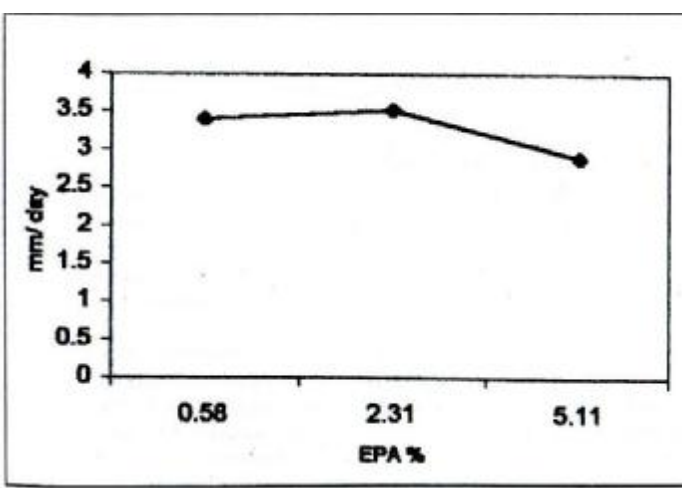

Fig. 2. Effect of different dietary levels of EPA on growth index of sea bass *D. labrax* larvae after 24 days from hatching.

markedly increased as the EPA levels increased (Figs. 1 and 2). The same results were obtained by Watanabe et al. (1989). They found that low growth and survival rates were noticed when red sea bream larvae were fed with EPA deficient diets and they were effectively improved by elevation of dietary EPA in their feed. Léger et al. (1987) found a linear relationship between the EPA content of *Artemia* and the biomass of *Mysidopsis bahia* to which freshly hatched *Artemia* were fed. Thus EPA (20:5 ω_3) is one of the most important ω_3 family that should be found in larval feeds with appropriate levels (Takeuchi et al. 1990; Rodriguez et al. 1993; Rainuzzo et al. 1997).

Conclusion

Optimal utilization of microalgae in the first feeding of marine fish may enhance the success of the larval rearing process through long term enrichment technique of rotifers. The fatty acid composition of rotifers was reflected in the corresponding fatty acids of algae they fed on. Therefore, a good selection of algal species rich in n_3 may cover the n_3-HUFA requirements of sea bass larvae that is important to obtain the high survival, growth and quality of larvae. *Tetraselmis* was found to be a good source for rotifers feeding than *Chlorella* that is commonly used in Egyptian marine hatcheries due to its higher EPA content.

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