Aquaculture, 49 (1985) 223—229
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

PRELIMINARY RESULTS ON THE NUTRITIONAL EVALUATION OF ω3-HUFA*-ENRICHED *ARTEMIA* NAUPLII FOR LARVAE OF THE SEA BASS, *DICENTRARCHUS LABRAX*

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(Accepted 27 June 1985)

ABSTRACT

Van Ballaer, E., Amat, F., Hontoria, F., Léger, P. and Sorgeloos, P., 1985. Preliminary results on the nutritional evaluation of ω3-HUFA-enriched *Artemia* nauplii for larvae of the sea bass, *Dicentrarchus labrax*. *Aquaculture*, 49: 223—229.

Freshly hatched San Pablo Bay and Great Salt Lake *Artemia* nauplii (ω3-HUFA-poor) and enriched meta-nauplii (ω3-HUFA-enriched) were compared to Reference *Artemia* nauplii (ω3-HUFA-rich) as a food source for the larvae of the sea bass, *Dicentrarchus labrax*. Significant differences in survival and biomass production between the treatments could be attributed to the ω3-HUFA content of the food. A delayed mortality in fish larvae fed the Great Salt Lake *Artemia* is suspected to be caused by a high level of α-BHC in this *Artemia* source. Growth was not significantly different between the treatments, most probably due to the large variation in growth rate within the treatments, as well as the low number of surviving fish in some treatments.

INTRODUCTION

Growth and survival of marine fish larvae are often influenced by the size and/or the dietary value of the organisms used as food (Sorgeloos, 1981a). It is well known that not all strains of *Artemia* guarantee equal culture success in aquaculture hatcheries (Léger and Sorgeloos, 1984). Recent investigations have indicated that substantial amounts of essential fatty acids, i.e. the highly-unsaturated fatty acids 20:5ω3 and 22:6ω3, largely determine the nutritional effectiveness of *Artemia* nauplii and of *Brachionus* as food for larval marine finfish (e.g. Watanabe et al., 1978; Fujita et al., 1980) and crustaceans (Léger et al., 1985a, b). Nutritional tests with larval sea bass, *Dicentrarchus labrax*, fed with enriched rotifers *Brachionus plicatilis* (Gatesoupe and Luquet, 1981; Gatesoupe and Robin, 1982) or with enriched *Artemia* nauplii (Robin et al., 1981) have demon-

*Highly unsaturated fatty acid.

strated that the use of fortified diets in the hatchery can markedly improve sea bass culture. None of these studies, however, provided analytical data on fatty acid levels in the larval food, before and after enrichment with HUFA's.

The present experiment was designed to evaluate ω3-HUFA-poor *Artemia* prefed on a ω3-HUFA-rich diet as food for the larvae of *Dicentrarchus labrax*.

METHODS AND MATERIALS

Artemia cysts from San Pablo Bay, California, U.S.A. (SPB 1628) and from the North Arm of the Great Salt Lake, Utah, U.S.A. (GSL NA) were selected as ω3-HUFA-poor *Artemia* sources (Schauer et al., 1980; own results). Reference *Artemia* cysts (RAC) were used as a positive control for ω3-HUFA-rich *Artemia* (Sorgeloos, 1981b). Cysts were incubated in natural seawater (38 ppt) under continuous aeration and artificial illumination. After harvest at T90 hours (Vanhaecke and Sorgeloos, 1982; see Table 1), instar I nauplii were separated from the hatching debris and transferred to fresh seawater. Enriched *Artemia* nauplii were prepared as follows: after the HUFA enrichment product AA18 (Léger et al., 1985a) had been homogeneously mixed with the dry cysts at a ratio of 1:5 they were incubated in natural seawater under the same conditions as mentioned above. In order to provide sufficient time for bioencapsulation of the enrichment diet in the *Artemia* meta-nauplii, the incubation time was prolonged for 24 h upon instar I nauplii production, i.e. T90 + 24 h (see Table 1).

TABLE 1

Experimental treatments and hatching conditions for the production of instar I and enriched *Artemia* nauplii

Treatment	Abbreviation	Density (g product/l)	Temperature (°C)	Incubation time (h)
San Pablo Bay 1628 instar I	SPB I	2	25	22
Great Salt Lake North Arm instar I	GSL I	2	25	22
San Pablo Bay 1628 + AA18 enrichment diet	SPB ED	3	30	46
Great Salt Lake North Arm + AA18 enrichment diet	GSL ED	3	30	46
Reference *Artemia* cysts instar I	RAC	2	25	26

Enriched meta-nauplii were harvested from the hatching debris and thoroughly rinsed in order to remove the non-consumed food particles.

In the culture tests with the different *Artemia* preparations 480 *Dicentrarchus* larvae were used. These larvae had been fed on *Brachionus plicatilis* from day 3 to day 37 after hatching. Rotifers were reared on baker's yeast and enriched with *Platymonas* sp. prior to their transfer to the fish tanks. At day 37 *Dicentrarchus* larvae were transferred into 2 l cylinders, exposed to natural light conditions, and stocked with 10 larvae per l (4 replicates per treatment). Culture water (salinity 38 ppt, 18—23°C, pH = 8.3) was renewed at a flow rate of 2 l h^{-1}.

Sea bass larvae were fed twice a day (morning and evening) at the beginning of the experiment and once a day towards the end. After the first feeding, *Artemia* nauplii were stored at 4°C (Léger et al., 1983). The initial density of one nauplius per ml was adjusted daily in order to ensure ad-libitum feeding of the larvae. Prior to feeding, dead fish larvae, debris and non-consumed *Artemia* were removed. Survival was recorded daily, and after 19 days on the *Artemia* diet individual length and wet weight were determined. Biomass production was calculated from the multiplication of individual wet weight by percent survival. Data were treated statistically in a one-way analysis of variance, and Duncan's multiple range test was used to determine significant differences among means. Prior to analysis, survival data were normalized through arcsin $\sqrt{\%}$-transformation (Snedecor and Cochran, 1967).

The fatty acid profile of freshly hatched and enriched *Artemia* was determined. After homogenization with an ultrasonic homogenizer, lipid extraction, saponification and esterification were done according to the procedure described in Schauer and Simpson (1978). Fatty acid methyl esters were injected on a capillary column (25 m fused silica, 0.32 mm I.D.; liquid phase, Silar 10C; film thickness, 0.3 μm) installed on a Carlo Erba Fractovap 2330 gas chromatograph. Operating conditions were as follows: solid injector; H_2 carrier gas at a flow rate of 1.9 ml min^{-1}; F.I.D. detection; oven temperature programme, 154°C to 200°C at 1.5°C min^{-1}. Peak identification and quantification was done with a calibrated plotter-integrator (Hewlett Packard 3390A). The internal standard procedure, with 20:2ω6 as an internal standard, was used for quantitative analysis.

RESULTS

Fatty acid analysis (Table 2) revealed considerable differences in 20:5ω3 content between instar I nauplii in San Pablo Bay, Great Salt Lake and reference *Artemia*. After enrichment of the former strains, 20:5ω3 concentrations increased and substantial levels of 22:6ω3 were detected; total ω3-HUFA contents in enriched *Artemia* approximated the level found in reference *Artemia*.

Survival and biomass production of larval *Dicentrarchus labrax* showed

TABLE 2

Fatty acid methyl esters (FAME) in freshly-hatched and enriched *Artemia* nauplii (mg fatty acid methyl ester per g *Artemia* dry weight; abbreviations as in Table 1)

FAME	RAC	GSL I	SPB I	GSL ED	SPB ED
20:5ω3	10.6	0.3	0.5	3.4	2.2
22:6ω3	—	—	—	2.3	3.1
ω3-HUFA[1]	11.2	0.5	2.6	6.7	9.6

[1] Total amount of ω3-highly unsaturated fatty acids (20:3ω3, 20:4ω3, 20:5ω3, 22:3ω3, 22:4ω3, 22:5ω3, 22:6ω3).

TABLE 3

Survival, mean length, mean wet weight and biomass production data for larval *Dicentrarchus labrax* after 19 days of *Artemia* feeding (abbreviations as in Table 1)

	SPB I	GSL I[1]	SPB ED	GSL ED	RAC
Survival (%)	15.6[c]	1.4	93.0[a]	66.5[b]	92.3[a]
SD	4.2	—	6.3	12.1	5.5
Mean length (mm)	18.2[a]	20.9	19.6[a]	18.2[a]	19.4[a]
SD	1.6	—	1.3	1.8	0.9
Mean wet weight (mg)	43.8[a]	52.0	60.5[a]	50.0[a]	58.0[a]
SD	11.8	—	12.9	11.8	7.6
Biomass prod. (mg %)	653.9[c]	72.8	5652.3[a]	3402.5[b]	5323.0[a]
SD	101.2	—	1346.8	1260.4	435.4

[1] Only one larva left at day 56; GSL I data are therefore not included in statistical analysis.
SD: standard deviation.
[a,b,c]Means with different superscripts are significantly different ($P = 0.05$).

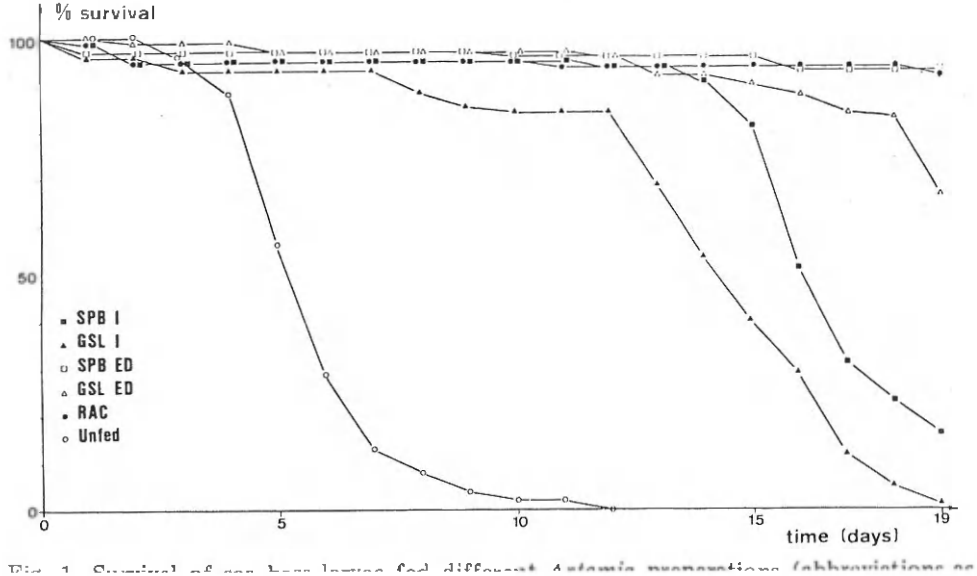

Fig. 1. Survival of sea bass larvae fed different *Artemia* preparations (abbreviations as

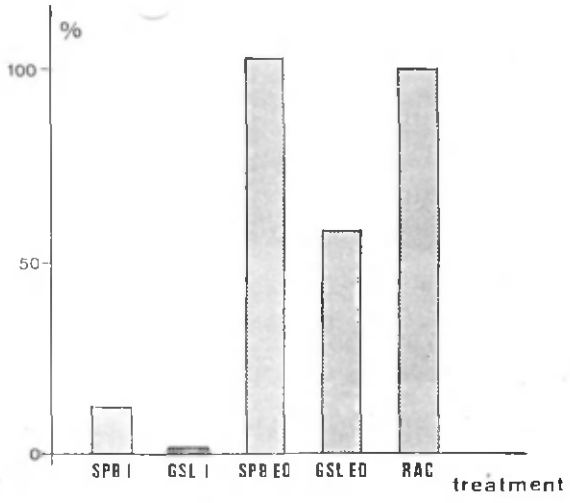

Fig. 2. Biomass production of sea bass larvae fed different *Artemia* preparations (expressed in % of the RAC treatment; abbreviations as in Table 1).

substantial differences between the enriched and non-enriched treatments (Table 3; Figs. 1 and 2). A considerable improvement was noticed after prefeeding SPB and GSL *Artemia* with the ω3-HUFA-rich diet AA18, i.e. RAC and enriched SPB treatments gave equal biomass yields. Biomass production in the GSL-enriched treatment was lower due to increased mortalities at the end of the test (Fig. 1). Although mortalities started only later, reduced activity of the sea bass larvae was noticed by day 7 and day 10 for the instar I treatments with GSL and SPB *Artemia*, respectively. Unlike the marked differences in survival rates, growth differences (e.g. mean length or weight) among the different treatments were not significant.

DISCUSSION

The results obtained in this study confirm the different nutritional value of various *Artemia* strains as food for larval marine fish. Furthermore, additional evidence is provided that the dietary value of *Artemia* can be correlated with differences in ω3-HUFA levels in the nauplii.

Although the impact of essential fatty acids is most pronounced on larval survival, its effect on growth appears to be minimal. This should not be generalized since our length and weight data have little statistical value, as a result of large variations in growth within treatments, as well as a low level of survival in some treatments. New experiments are needed, with larger numbers of animals per treatment.

Towards the end of the test period a significant mortality occurred in the ω3-HUFA-enriched GSL treatments. Aside from nutritional differences, the three *Artemia* strains used here varied also in their levels of contamina-

tion, the most important difference being the high level of the pesticide residue α-BHC in the GSL NA *Artemia* (Olney et al., 1980; K. Simpson, personal communication, 1984). Pesticide accumulation in the sea bass larvae might explain the mortality in the enriched GSL treatment as well as the earlier mortality in fish fed GSL than in those fed SPB instar I nauplii.

These results, which should be verified in new culture tests of longer duration, indicate that ω3-HUFA content has only a partial role in the determination of the nutritional value of *Artemia*, at least when using the *Artemia* strain from the Great Salt Lake, North Arm, as food for marine fish larvae.

ACKNOWLEDGEMENTS

This study was supported by the Belgian National Science Foundation (NFWO) grant FKFO 32.0012.82 and by the US-Environmental Protection Agency grant CR 811042-02-0. Culture facilities were made available at the Instituto de Acuicultura de Torre de la Sal.

Patrick Sorgeloos is a senior scientist with the NFWO. International Study on *Artemia* contribution number 34.

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