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COMPARISON OF THE FATTY ACID PROFILE OF WILD CAUGHT FINGERLINGS AND YOLK SAC SEA BASS *(DICENTRARCHUS LABRAX)* LARVAE WITH CULTURED HEALTHY LARVAE AND LARVAE SUFFERING FROM WHIRLING DISEASE

by

SERGE CORNEILLIE*, CARMELO AGIUS** and FRANS OLLEVIER*

*Laboratory for Ecology and Aquaculture,
Zoological Institute, Catholic University of Leuven,
Naamsestraat 59, 3000 Leuven (Belgium)

**Faculty of Science, Kingston Polytechnic,
Penzhyn Road, Kingston-Upon-Thames, Surry KII 2 EE, Great-Britain

SUMMARY

Analysis of the fatty acids of total lipids of wild fingerlings (1-4 g) of sea bass (*Dicentrarchus labrax*) caught off the north coast of France shows that more than 30 % of total fatty acids consists of the two essential fatty acids eicopentaenoic (C20:5 n-3) and docosahexaenoic (C22:6 n-3) acids. One- and three-day old yolk sac larvae have even higher amounts of these two important fatty acids (about 40 %). On the other hand, laboratory-reared 60-day old larvae had much lower concentrations of C20:5 n-3 (4 % of total fatty acids) and C22:6 n-3 (5.5 % of total fatty acids). In larvae suffering from whirling or spinning disease the percentage of C20:5 n-3 was only 2.8 % and only traces of C22:6 n-3 were found. These results emphasize the importance of these fatty acids for cultured sea bass larvae.

Key-words : HUFA, Sea-Bass, Larvae.

INTRODUCTION

Highly Unsaturated Fatty Acids (HUFA's, fatty acids with at least 20 Carbons and 3 unsaturated bonds) are very important components of biological membranes. Fresh water fishes are able to synthesize them starting from lenoleic (C18:2 n-6) and lenolenic (C18:3 n-3) acid. Marine fish are incapable of de novo synthesis of HUFA's such as C20:5 n-3 and C22:6 n-3 and dietary sources of these fatty acids are therefore essential for normal growth and development (WATANABE, 1982). During the last decade many studies have demonstrated the importance of HUFA's especially C20:5 n-3 and C22:6 n-3 for the culture of marine fish larvae and shrimps

(FUJITA *et al.*, 1980; HALVER, 1980; SCHAUER *et al.*, 1980; WATANABE, 1982; LEGER and SORGELOOS, 1984; LEGER *et al.*, 1985; SUZUKI et al., 1986; DENDRINOS and THORPE, 1987; CORNEILLIE, 1989). To date the rearing of marine fish larvae still requires the use of live foods. Most intensively used are the rotifer *Brachionus plicatilis* and the crustacean *Artemia* sp... However a lot of studies showed that these organisms are very low in C20:5 n-3 and/or C22:6 n-3 (WATANABE *et al.*, 1980, 1982, 1983; VAN BALLAER *et al.*, 1985). Many investigators found higher survival rates and better morphologically developed larvae when the live prey were enriched with HUFA's (bio-encapsulation) just before administration to the marine fish larvae (WITT *et al.*, 1984; FRANICEVIC *et al.*, 1987; KITAJIMA, 1987; WALFORD and LAM, 1987).

Despite the awareness of the importance of C20:5 n-3 and C22:6 n-3 for the culture of sea bass larvae, no one has ever studied the fatty acid profile of wild caught juveniles, which should be indicative of optimal requirement in culture. The present study was undertaken to compare the fatty acid profiles of wildcaught and cultured fish as well as to establish whether whirling disease can be caused by fatty acid inadequacy.

MATERIALS AND METHODS

Collection and culture of sea bass larvae

Wild fingerlings (1 to 4 g, about 4-5 months old) were caught by nets at the end of April 1987 from the estuary of Ambleteuse (north France). After transporting them live to the laboratory 5 fingerlings were frozen for analysis.

Newly hatched larvae obtained from C.O.B. (Brest, France) in May 1987 were put in larval tanks of 100 litres. The larvae were cultured at Leuven under normal conditions (Corneillie *et al.*, 1989). Larvae were fed with *Brachionus plicatilis* enriched with SELCO (Artemia Systems N.V., Ghent; day 5-13), nauplii of *Artemia* (day 10-20); metanauplii of *Artemia* enriched with SELCO (day 20-45), and granules 000 (Trouvit, Trouw Ghent, from day 43 on).

Five samples of 1- and 3-days old yolk sac larvae were collected and frozen for analysis; each sample contained 150-200 larvae. At day 60, five samples of larvae each containing 3 fish were collected. Forty days old larvae suffering from whirling disease were also collected. All the samples were frozen directly in liquid nitrogen and stored in a freezer at $-$ 70° C until analysis.

Fatty acid analysis.

Total lipids were extracted by homogenising whole fish with chloroform-methanol (2:1 V/V) (FOLCH *et al.*, 1957). Fatty acid methyl esters were prepared by transesterification using a 1 % methanolic solution of sodium methoxide. The esters were injected on to a WCOT capillary column (25 m × 0.22 mm ID fused silica, CP SII-88, stationary phase, 0.2 micron film thickness) installed in a Sigma B

Perkin Elmer gas chromatograph, using hydrogen as carrier gas, split injection and the oven temperature programmed from 140° C to 220° C at 4° C/min. Peak identification and quantification was done with a calibrated plotter-integrator Perkin Elmer 3600 data station.

RESULTS

Table 1, reveals considerable differences in the fatty acid profile of total lipids of sea bass larvae from different origins. In wild caught fingerlings the fatty acid composition shows, on average, 35 % saturated fatty acids (primarly C16:0), 17 % monoenes (C16:1 and C18:1 isomers), and almost 35 % HUFA's (mainly C20:5 n-3 (16.4 %) and C22:6 n-3 (16.3 %)). The fatty acid composition of 1- and

TABLE 1

Fatty acid composition of total lipids from larvae of sea bass from different origins (expressed as area percentage of total fatty acids)

	Wild	*Yolk Sac*		*Leuven*	
		(1 day)	(3 day)	Healthy	Whirling
C14	3.3	3.4	1.7	2.9	0.8
C16	24.4	17.1	14.3	22.2	17.1
C16 : 1w7	6.2	10.2	10.0	3.1	1.0
C16 : 1w9					
C18 : 0	7.0	4.5	2.9	6.8	7.8
C18 : 1w9	11.1	15.4	17.6	12.6	17.8
C18 : 1w7					
C18 : 2w6	(tr)	3.0	5.8	15.7	18.2
C18 : 3w3	(tr)	(tr)	(tr)	2.0	10.3
C20 : 5w3	16.4	11.0	10.2	4.0	2.8
C22 : 4w6	(tr)	(tr)	(tr)	1.7	(tr)
C22 : 4w3					
C22 : 5w3	0.3	(tr)	1.4	0.7	(tr)
C22 : 6w3	16.3	24.0	25.7	5.5	(tr)

(Wild : wild caught larvae ; Yolk sac : 1- and 3-day old yolk sac larvae ; Leuven : larvae cultured in Leuven ; Whirling : larvae suffering from whirling disease ; (tr) : traces ; values are means of 3 to 5 replicates and the difference between the replicates is less than 5 % ; Cn : XwY : Cn number of C-atoms, X number of double bonds, Y position of the first double bond starting from the methyl end.

3-days old yolk sac larvae shows on average 22 % saturated fatty acids, 26 %monoenes and 40 % HUFA's. The yolk sac larvae have very high amounts of C22:6 n-3 (60 % of total HUFA's).

Laboratory cultured larvae had nearly the same HUFA content of wild larvae ; however the percentage of C20:5 n-3 (4 %) and C22:6 n-3 (5.5 %) was rather low. These larvae had, on the other hand, high concentrations of linoleic acid (C18:2 n-6), a non-essential fatty acid for marine fishes.

Larvae suffering from whirling disease had very low concentrations of C20:5 n-3 (2.8 %) and only traces of C22:6 n-3. The proportions of linolenic acid (C18:3 n-3) at 10.3 % and linoic acid (C18:2 n-6) at 18.2 % are high when compared to the other groups.

DISCUSSION

The « probable » fatty acid requirement of sea bass larvae was assessed by analysis of the fatty acid content of yolk sac larvae and wild caught fingerlings. The fatty acid profile of the total lipids emphasise the importance of eicosapentaenoic (C20:5 n-3) and docosahexaenoic (C22:6 n-3) acids. Ten to 16 percent of total fatty acid consists of C20:5 n-3 in both groups and the percentage of C22:6 n-3 is even higher, 16 to 26 %. In 1-day old yolk sac larvae of herring (*Clupea harengus*) it is also these two HUFA's which seems to be important (6.8 and 15.6 % respectively, table 2) (TOCHER *et al.*, 1985). TOCHER and SARGENT (1984) also analysed ripe roes of different Northwest European marine fishes (cod, *Gadus merlangus*). About 70 % of the lipids were formed by the polar classes (mainly phosphatidylcholine and phosphatidylethanolamine). Analysis of the fatty acid of total phospholipids showed the importance of C20:5 n-3 (12 to 15 %) and C22:6 n-3 (28 to 31 % in all the analysed fish, 8 species). Also DENDRINOS and THORPE (1987) estimated the probable fatty acid requirement of larval Dover sole (*Solea solea*) by analysis of the fatty acid content of the yolk of sole eggs. Again C22:6 n-3 seems to be very important (21.6 %) ; however C20:5 n-3 represented only 4.6 % of total fatty acid.

As has already been mentioned FRANICEVIC *et al.* (1987) found much better survival and growth of sea bass larvae when *Artemia* metanauplii were enriched with oils containing high amounts of HUFA's. The same findings were reported for sole larvae (DENDRINOS and THORPE, 1987). WITT *et al.* (1984) also found better performance of turbot larvae (*Scophtahlmus maximus*) when they were fed with copepods, richer in HUFA content than *Artemia*. WATANABE *et al.* reported (*) poor growth and heavy mortalities of red sea bream larvae (*Pagrus major*) when they were fed rotifers containing a low percentage of n3-HUFA's. All these results confirm the importance of n3-HUFA's for marine fish larvae, not least sea bass.

(*) « International symposium on feeding and nutrition in fish », Bergen, Norway, August 23-27, 1987.

TABLE 2

Fatty acid composition of total lipids from yolk sac larvae of sea bass and herring and yolk of fertilised eggs of sole (expressed as area percentage of total fatty acids)

	Yolk sac larvae (1 day old) of		*Yolk of*
	Sea bass	Herring *	Sole #
C14	3.4	5.3	2.8
C16	17.1	21.8	19.9
C16 : 1w9	10.2	—	8.7
C16 : 1w7		6.9	—
C18 : 0	4.5	2.5	2.9
C18 : 1w9	15.4	18.9	13.1
C18 : 1w7		6.1	3.7
C18 : 2w6	3.0	1.3	1.7
C18 : 3w3	(tr)	1.4	0.9
C20 : 5w3	11.0	6.8	4.6
C22 : 4w6	(tr)	—	—
C22 : 4w3			
C22 : 5w3	(tr)	0.9	—
C22 : 6w3	24.0	15.6	21.6

((tr) : traces ; — : not detected ; — values are means of 3 to 5 replicates).

* TOCHER *et al.*, 1985

\# DENDRINOS and THORPE, 1987.

The results of the fatty acid analysis of the laboratory-reared larvae demonstrate the low percentage of these two essential fatty acids. In the healthy larvae the percentage of C20:5 n-3 and C22:6 n-3 was only 10 % of total fatty acids ; this is very low compared to the 33 % found in wild caught sea bass larvae. The low survival of the larvae found in our experiments (12 % at day 45) could be caused by this low HUFA content.

During this expirement, we lost a part of the larvae (about 25 %) due to a whirling or spinning disease. The fatty acid profile of these larvae showed the virtual absence of C22:6 n-3 and the low percentage of C20:5 n-3 (2.8 %). The percentage of linoleic and linolenic acid (18.2 and 10.3 % respectively) was high compared to that of the wild caught and yolk sac larvae. The percentage of linoleic acid was also high in healthy laboratory-reared larvae (15.7 %).

These low concentrations of the n3-HUFA's are hard to explain because the larvae were fed with live prey enriched with SELCO. The SELCO (a special diet rich

in HUFA's) is taken up by the zooplankton and stocked in the intestine (bio-encapsulation). When the fish larvae take up the zooplankton organisms, they also profit from the enrichment. A possible explanation for the low HUFA-concentration in the laboratory-cultured (whirling) larvae could be that HUFA-enriched prey were only administered once a day, because of the low density of the larvae at the end of the experiment. In addition, the live prey that were not eaten within the first hours, lost their gutcontent by normal excretion processes. By this, the effect of the enrichment disappears. Therefore it is better to feed larvae two or three times a day with freshly enriched prey. It must also be noted that larvae suffering from whirling disease had very little intestinal contents (90 % of these larvae had an empty or very poorly filled intestine). Loss of appetite could cause a dramatic decrease in the HUFA-content. Other research groups found that spinning larvae were infected with a Birna-virus (BONAMI *et al.*, 1983). In France, they also found VHS-virus in sea bass and turbot (HILL, 1986). However, there is no direct evidence to connect virus infection and whirling disease. In the present study fairly extensive virological analysis using tissue culture techniques failed to reveal any viruses in any of the larvae.

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