Aquaculture
Volume 267, Issues 1-4, 3 July 2007, Pages 165-174
doi:10.1016/j.aquaculture.2007.01.031

Is it possible to influence European sea bass (*Dicentrarchus labrax*) juvenile metabolism by a nutritional conditioning during larval stage?

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Abstract:

The purpose of this study was to check if it is possible to influence sea bass juvenile metabolism by a conditioning of larvae from day 6 post hatching to day 45 to a low or a high HUFA compound diet (LH, 0.8% EPA + DHA and HH, 2.2% EPA + DHA) when reared at 16 or 22 °C. Following a 3-month intermediate period (at 19 °C using a commercial diet), the adaptability of the 4 initial larval groups to a HUFA experimental deprived diet (0.5% EPA + DHA) were tested at 19 °C in a 60 day-experiment (d-151–211). The four experimental duplicated conditions were ex-LH16 and ex-HH16 for the 2 groups previously reared at 16 °C (initial weight, 7.3 ± 0.5 g) and ex-LH22 and ex-HH22 for the 2 groups previously reared at 22 °C (initial weight, 11.1 ± 0.5 g). Survival was maximal and there was a 1.6–2 fold increase in mass during the experiment. Growth was similar in the 4 experimental groups: NS difference in growth curve slopes (P = 0.7). At the end of the experiment (d-211), whole body fat levels were in the same range in all groups (13–15% WW). The fatty acid (FA) composition in polar lipids (PL) and total lipids (TL) were significantly affected by initial weight related to larvae conditioning, which can be mainly attributed to a dilution effect (impact of initial FA content on final FA content versus relative mass increase during the course of the experiment). Conversely to this trend, DHA content in PL was higher in the ex-LH groups than in the ex-HH groups whatever thermal conditioning of larvae was. This indicated that ex-LH groups had a better capacity to adapt to a deficient HUFA diet than ex-HH fish. The relative expression of the delta-6 desaturase (Δ6D) was significantly higher in ex-LH than in ex-HH groups (P < 0.001) between d-151 and d-181, which suggested that Δ6D transcription in ex-LH groups was positively modulated by the HUFA-deprived diet. This stimulation of the first step of the desaturation/elongation pathway could allow synthesizing FA needed to compensate low dietary HUFA supply. This study shows for the first time that it seems possible to influence juvenile fish metabolism by a nutritional conditioning during the larval stage.

Keywords: Aquaculture; Delta-6 desaturase; Dicentrarchus labrax; HUFA; Programming concept

1. Introduction

In contrast to freshwater fish, marine fish require the presence of preformed highly unsaturated fatty acids (HUFA) in their diet as they have a low capacity to bioconvert 18 carbon fatty acids (linoleic 18:2n-6 and linolenic 18:3n-3) into HUFA with 20 or 22 carbons (arachidonic 20:4n-6, eicosapentaenoic EPA 20:5n-3 and docosahexaenoic acid DHA 22:6n-3), (Kanazawa et al., 1978; Mourente and Tocher, 1994). The first step of this bioconversion requires the presence of the delta-6 desaturase gene (Δ6D), which has been cloned in several freshwater species such as zebrafish (**AF309556**), common carp (**AF309557**), rainbow trout (Seiliez et al., 2001). Δ6D gene has also been cloned in two marine fish species: gilthead seabream and turbot (Seiliez et al., 2003; Zheng et al., 2004). In gilthead seabream, an enhanced expression of the gene was obtained by feeding juveniles a HUFA-free diet. It has been previously showed by Cho et al. (1999) and Seiliez et al. (2001) that dietary HUFA inhibits Δ6D gene expression in mammals and rainbow trout. The deficiency in Δ6D activity observed in marine fish can be related to the abundance of n-3 HUFA in the marine food chain, which has induced an adaptation (Sargent et al., 1995) or a repression of desaturase activity (Olsen et al., 1990).

Until now, the main difficulty to meet the dietary n-3 HUFA requirement occurred in larvae fed living prey (Sargent et al., 1999). Specifically EPA and DHA are required for growth and play a major role in vision and brain development (Bell et al., 1986; Sargent et al., 1997). As long as fish oil and fish meal represent the primarily ingredients of aquafeeds, juvenile HUFA requirements are easily covered. However, the rapid increase in farmed fish production in addition to the decline in natural stock has lead to look for substitutes for fish products commonly used in aquafeeds (Lodemel et al., 2001; Ringo et al., 2002). Incorporation of vegetable feedstuffs in fish feeds constitutes at the present time, the only solution in Europe, although these vegetable feedstuffs do not contain adequate n-3 HUFA to cover marine fish requirements except C18 HUFAs which may disturb fish physiology (Bell et al., 1996; Parpoura and Alexis, 2001). In European sea bass juveniles, the minimal dietary n-3 HUFA requirement to sustain maximum growth is 0.7% dry matter (DM; Skalli and Robin, 2004), indicating that only some marine feedstuffs (fish meal or fish oil) can fulfil this requirement. There is also considerable interest in producing fish selected for their capacity to use vegetable feedstuffs as incorporation of these feedstuffs in high proportion in feeds usually lead to a decrease in fish dietetic quality expressed in terms of n-3 HUFA flesh content (Regost et al., 2003). This could be possible by applying a metabolic programming, using a nutritional and thermal conditioning during the young stage, as described in mammals (Lucas, 1998). The concept of metabolic programming is defined as an adaptive process at the cellular, molecular or biochemical level occurring during very young stages of organism development and which durably modify the genomic expression in the adult. In the rat, it has been shown that nutritional and thermal conditioning during young stages had consequences on survival, growth, learning process, lipid and glucoid synthesis (Lucas, 1998). In marine fish, several studies have shown the importance of temperature and trophic conditions on larval development, maturation of digestive functions, growth and larval quality (Bergeron and Person-Le Ruyet, 1997; Koumoundouros et al., 2001; Zambonino and Cahu, 2001; Cahu et al., 2003; Lopez-Albors et al., 2003; Robin and Vincent, 2003). Since this adaptive process occurs during ontogenesis, the adaptability of juveniles could be modulated by larval "history".

The aim of this study was to determine if it is possible to influence European sea bass juvenile metabolism by a nutritional (HUFA) and thermal conditioning during larval development and specially if it is possible to modulate the Δ6D expression.

2. Materials and methods

2.1. Rearing conditions and experimental design

Replicated groups of European sea bass, *Dicentrarchus labrax*, larvae were initially reared at 16 or 22°C and fed microparticulated diets from mouth opening (day 6 post-hatching, d-6), with a low (LH) or high (HH) HUFA content (0.8 and 2.2% EPA+DHA on dry matter basis, respectively). The four experimental conditions applied to larvae up to day 45 (d-45) were LH16, HH16, LH22 and HH22. The larval period was followed by an intermediate period of three months (d-46 to d-141) during which the four groups were separately held at 19°C and fed a commercial diet with 2.7% EPA+DHA (DM basis).

At the end of this initial conditioning period, juveniles of each group were anaesthetised (ethylene-glycol-monophenylether, 0.15‰), individually weighed and then fish around the modal class were selected. At d-141, graded-fish were randomly distributed in square tanks, with an effective

volume of 60 l, in order to obtain a similar biomass per condition: 5 tanks per condition with 75 fish per tank for the initial 16°C groups (ex-HH16 and ex-LH16) and 40 fish per tank for the initial 22°C group (ex-HH22 and ex-LH22). They were acclimated in the experimental unit for 10 days at the same temperature and diet as for the intermediate period (19°C; commercial 2.7% EPA+DHA diet).

At d-151, 4 tanks per group were supplied with an experimental HUFA deprived diet (0.5% EPA+DHA, Table 1) and the last one was fed as previously in order to evaluate, from a qualitative point of view, the delay in growth induced by the deprived diet (this group was named qualitative reference, QR). Fish were hand fed to visual satiation at 09:00 and 15:00 h in order to avoid feed waste. All tanks were supplied with running seawater at 19°C, 34.5‰, 12L:12D light cycle. Water flow rate was 600% per h, which stabilized oxygen concentration around 90±5% saturation and prevented ammonia accumulation. The experiment lasted until the fish final weight was at least twofold the initial weight (2 months, d-211).

2.2. Sampling procedures

Fish were fasted for 12 h and water volume was lowered prior to random sampling using an appropriate net.

To measure Δ6D gene expression, 9 fish per experimental condition (3 fish x 3 tanks) were taken at d-151, d-181 and d-211, immediately frozen in liquid nitrogen and stored at –80°C until assayed. The expression of Δ6D (accession number: **AJ715505**) was measured in livers.

For body composition and lipid analysis, additional fish were taken at the start and end of the experiment (d-151 and 211); they were previously anaesthetised (ethylene-glycol-monophenylether 0.15‰), individually weighed, frozen in liquid nitrogen and stored at –80°C pending analysis. 8 fish for ex-16 groups and 6 fish for ex-22 groups were taken from 2 tanks per experimental condition at d-151, and from all tanks per condition at d-211 (n=4 tanks per experimental condition).

Growth was estimated by weighing individually all anaesthetised fish every two weeks (± 0.1 g). Growth index and feeding parameters were calculated for the 60-day-experiment (d-211) as follow:

$$SGR_{d\text{-}151\text{-}d\text{-}211} = 100 \times (\ln w_{d\text{-}211} - \ln w_{d\text{-}151}) \text{ day}^{-1} \quad (1)$$

where SGR in $\%.day^{-1}$, $w_{d\text{-}151}$ and $w_{d\text{-}211}$ are the initial and final mean body weights, respectively.

As some fish were regularly removed from the tanks, daily feed intake (FI) and apparent feed conversion ratio (FCR) were calculated taking into account the average mass for d-151-d-211 period, using the following expressions:

$$FI_{d\text{-}151\text{-}d\text{-}211} = 100 \times [\text{mean daily mass of dry feed ingested (g)} \times \text{mean wet fish mass}^{-1}\text{ (g)}] \quad (2)$$

where: FI in $\%.day^{-1}$, mean fish mass = (fish mass at d-211 +fish mass at d-151) 2^{-1}, fish mass = mean body weight x fish number.

$$FCR_{d\text{-}151\text{-}d\text{-}211} = \text{dry feed ingested (g)} \times \text{fish mass gain}^{-1}\text{ (g)} \quad (3)$$

2.3. Analytical methods

2.3.1 Δ6D gene expression

Dissections were conducted on a glass plate maintained at 0°C. The whole liver was extracted and the gall bladder was removed because it destructs RNAs. cDNAs were obtained in duplicate from total RNA by using a Quantitect Reverse Transcription kit with integrated removal of genomic DNA contamination (QIAGEN GmbH, Hilden, Germany). Real-time PCR was performed using the iCycler iQTM (Bio-Rad® Laboratories Inc.). Quantitative PCR analyses for each gene were performed in triplicate for each cDNA duplicate (6 assays for each studied gene per experimental group), in a total volume of 15µl containing 5 µl cDNA (dilution: 10-2), 0.5 µl primers (10 µmol/l), 7.5 µl 2X iQ SYBR Green Supermix (Bio-Rad®, Hercules, CA). The specificity of forward and reverse primers of Δ6D gene was checked by sequencing the amplicon (Table 2; Eurogentec, Labège, France). Thermal cycling was initiated with incubation at 95°C for 13.5 min for activation of the hot-start enzyme, iTaqTM DNA polymerase. After this initial step, 45 cycles of PCR were performed. Each PCR cycle consisted of heating at 95°C for 30 sec for denaturing, at 60°C for 1 min for annealing and extension. Cycle threshold values (CT) corresponded to the number of cycles at which the fluorescence emission monitored in real time exceeded the threshold limit. Standard curves were established for each gene

by plotting the CT values against the log10 of 5 different dilutions (in triplicate) of cDNA sample solutions. Real-time PCR efficiency E was determined for each gene from the given slopes in Bio-Rad® software, according to the equation 4:

$$E=10[-1/slope] \quad (4)$$

We calculated the relative expression ratio of each gene using REST® software (http://www.wzw.tum.de/gene-quantification/). Relative expression ratio for a considered gene is based on the PCR efficiency (E) and the CT of a sample versus the control (standard group), and expressed in comparison to the reference gene (elongation factor EF-1), according to Pfaffl's mathematical model (Pfaffl, 2001)

$$Ratio=[(E_{gene})^{CTgene(control-sample)}]/[(E_{EF1})^{CTEF1(control-sample)}] \quad (5)$$

In this study, HH22 was used as the standard group because it was close to the rearing conditions in a fish farm. Normalization relative to EF1 provided a widely applicable value for comparative studies of gene expression at the mRNA level seeing that its expression is constant during activation and proliferation of cells (Gause and Adamovicz, 1994).

2.3.2 Fatty acid composition

Whole frozen fish were homogenised rapidly with a Hobart® mixer in order to keep a low temperature and then, more accurately using a Polytron® (PT 2100 Bioblock®). A representative portion (~5g) was taken for lipid analysis and ~3g were taken for dry weight measurement (105°C in an oven for 24 h). Lipid analysis was conducted on triplicates at d-151 and on duplicates at d-211. Extraction of total lipids was done according to Folch et al*.* (1957), with chloroform being replaced by dichloro-methane. The separation of neutral and polar lipids was performed according to the procedure described by Juaneda and Roquelin (1985). The total lipid (TL) extracts were fractionated on silica cartridges (Sep-Pack, Waters®), neutral lipids (NL) were eluted by chloroform and polar lipids (PL) by methanol. Fatty acid methyl esters (FAME) of TL were prepared by saponification and then methylation while PL FAME were prepared by transmethylation with sulfuric acid in methanol. All FAME were separated by gas chromatography (Auto-system Perkin-Elmer® with a flame ionisation detector, BPX 70 capillary column: 25 m x 0.22 mm i.d. x 0.25µm film thickness; split-splitless injector, with helium as carrier gas). The injector and detector temperatures were, respectively, 220 and 260°C. Initial temperature of the oven was 50°C, which increased to 180°C by increment of 15°C min^{-1}, maintained for 5 min, and then increased to 220°C by increments of 3°C min^{-1}. Data acquisition and handling were carried out by connecting the GLC to a PE Nelson computer. Individual FAMEs were identified by comparing the retention times of authentic standard mixtures. The results of individual fatty acid composition were expressed as percent of total identified FAME.

Chemical analyses of feed were performed in triplicate for each sample according to AOAC (Association of Official Analytical Chemists, 1984) methods: ash (7 h at 550°C), crude fat (Folch et al., 1957), crude protein (Dumas method with an Elementary NA 2000®, N × 6.25).

2.4. Statistical analysis

The data are presented as mean ± S.E. of the replicate groups. Concerning the weight gain between d-151 and d-211, regression curves were determined for each condition and they were compared using two-way ANOVA (Statistica®). Effects of temperature and diet on growth indices were tested using two-way ANOVA (Statistica®). Statistical differences in gene expression between control and samples were evaluated in group means by randomisation tests (Pfaffl et al*.*, 2002) using REST® software. Two thousand random allocations were performed and significant differences were considered at $P<0.05$. Effects of temperature and diet on fatty acid composition were tested using two-way ANOVA (Statistica®). When significant interactions were observed, differences between means were compared by Newman-Keuls test, differences were considered significant at $P<0.05$.

3. Results

3.1. Growth performance

All groups of fish appeared healthy and survival was 100% at the end of the 60-day experiment (d-211).

The changes in mean weight versus time are shown in Fig. 1. There was a 1.6-2 fold increase in mass in the four experimental groups. Growth was linear as described by the equations reported in Fig. 1 legend and similar for all groups (NS differences observed between the curve slopes; P=0.7).

As shown in Fig. 2, SGR and daily FI for all the experimental periods were affected by fish initial mean weight (Fig. 2A and 2B): SGR and FI decreased as initial weight increased. In comparison with the qualitative reference group (QR group) maintained on a commercial diet, SGR and FI of the 4 experimental groups were lower (75 and 96% of the QR group, respectively). The FCR (Fig. 2C) was in the same range regardless of fish size, 1.5 ± 0.1, and it was higher than in the QR group (despite no comparison was possible).

3.2. Lipid and fatty acid composition

TL content of fish at d-211 was 12.9±0.6, 14.2±0.9, 14.8±0.9 and 15.1±0.6 % wet weight in ex-LH16 group, ex-HH16, ex-LH22 and ex-HH22, respectively. More than 90% of the TL was NL. Since fish TL and NL composition were comparable, NL profile is not presented in Fig. 3. Fig. 3A and 3B represents the PL and TL content of 4 polyunsaturated fatty acids at d-211. Linoleic acid (18:2n-6) content was higher in TL than in PL (9.3 ± 0.5%). Conversely, n-3 HUFA content was lower in TL than in PL.

In PL, the levels of n-3 HUFA and arachidonic acid (20:4n-6) were significantly higher in ex-22°C ($P<0.001$ and $P<0.01$ for DHA) than in the ex-16°C groups. Linoleic acid was significantly higher in the ex-16°C groups ($P<0.001$), a significant interaction ($P<0.05$) was induced by a lower value in the ex-LH22 than in the ex-HH22 group, while no significant difference occurred within the ex-16°C groups. The DHA content in PL was higher in the ex-LH than in the ex-HH groups ($P<0.05$).

In TL, the quantity of 18:2n-6 was significantly higher ($P<0.001$) in the ex-16°C groups, than in the ex- 22°C groups, and in the ex-LH groups than in the ex-HH groups ($P<0.001$). DHA and EPA were affected by initial rearing temperature with higher contents for fish reared at 16°C than at 22°C ($P<0.01$ and $P<0.001$, respectively), and also by larval diets with higher contents in the ex-HH than in the ex-LH groups ($P<0.001$). This effect of larval diet affecting DHA in TL was opposite to that observed in PL. No significant differences were observed in 20:4n-6 content among groups.

Fatty acid (FA) composition of d-211 juveniles fed the HUFA deficient diet clearly differed from initial fish composition as well as from the composition of the QR group (at d-211) fed the commercial diet. These results are not reported in this study.

3.3. Δ6D gene expression

No amplification products were obtained in the negative control reactions (Fig. 4).

Between d-151 and d-181 of the experiment, the Δ6D expression significantly increased in groups fed the LH diet during the larval stage by a factor of 2 in the ex-LH16 group and 4 in the ex-LH22 group ($P<0.001$). In the ex-HH16 and ex-HH22 groups, this gene expression did not vary significantly with a factor of -1.2 and 1.7, respectively. Between d-151 and d-211, the Δ6D expression was not stimulated in any group with a factor of -7.61, 1.09, 1.09 and -1.19 in the ex-HH16, ex-HH22, ex-LH16 and ex-LH22 groups, respectively.

4. Discussion

This study brought new findings on the possibility to influence European sea bass juvenile metabolism by a nutritional conditioning during the larval stage. The nutritional conditioning was used to apply a metabolic programming, which is defined as an adaptive process at the cellular, molecular or biochemical level occurring during very young stages of organism development and, which durably modify the genomic expression in the adult. The strategy used in this study was to feed sea bass larvae a high or a low-HUFA diet at two temperatures, and an acclimation period to test the juveniles capacity to adapt to a HUFA-deprived diet (0.5% EPA+DHA). The HUFA-deprived diet used in this study was designed to be just below the requirement for n-3 HUFA (0.7% DM in 14g sea bass; Skalli and Robin, 2004).

Growth of sea bass juveniles was not affected by the larval conditioning, despite a high difference in initial weight resulting from rearing temperature and HUFA content in diets during the larval stage (Vagner et al., unpublished results). Although weight increase was acceptable as it doubled during the 60-days experiment (Skalli and Robin, 2004), it was affected by the HUFA-restricted diet: in all experimental groups, growth was about 25% lower than in the qualitative reference (QR) groups. In the QR groups, growth was in the normal range for sea bass of this weight class (Skalli and Robin, 2004). Under similar conditions, it has been showed by Skalli and Robin (2004) that a decrease in n-3 HUFA from 0.7% to 0.5% DM in the diet leads to a decrease in growth of 10%. In this study, the relative growth decrease was higher than expected, which can be partly explained by some differences in feed composition, for example in this study dietary protein and lipid content was slightly lower than in the diet used by Skalli and Robin (2004) (52 instead of 54% DM and 16.8 instead of 18.1% DM, respectively). A substitution rate of 50% of the main protein sources with lupin meal did not lead to an adverse effect on growth both in sea bass and turbot (Burel et al., 2000; Person-Le Ruyet et al., 2004). Our results showed that sea bass juveniles had a high capacity to adapt to a HUFA-deprived diet (0.5% DM), which is in agreement with previous studies (Person-Le Ruyet et al., 2004; Skalli and Robin, 2004).

This study showed that fish metabolism can be modulated by larval life history. The significantly higher Δ6D expression observed in the ex-LH groups than in the ex-HH groups indicated that FA desaturation was positively influenced in fish conditioned with a HUFA-deficient diet during larval stage. A Δ6D activity has been shown in gilthead seabream by Tocher and Ghioni (1999) despite it is generally very low in marine fish compared to salmonids (Kanazawa, 1985). A nutritional modulation of the Δ6D -like cDNA in a marine fish was demonstrated for the first time by Seiliez et al. (2003), who showed that the FA profile of gilthead seabream fed n-6 and n-3 FA deprived diets was characterized by high levels of Δ6D desaturation products (18:2n-9 and 20:2n-9), combined with a high expression of the Δ6D -like gene. The current study confirmed that it is possible to positively modulate the expression of this gene in sea bass juveniles fed a HUFA-deficient diet during larval development. Transcription of Δ6D gene is modulated by both peroxisome proliferators (PP) and sterol binding element protein-1 (SREBP-1a and SREBP-1c) (for review see Nakamura and Nara, 2002). SREBP-1 activates genes for FA synthesis in liver. Sterol regulatory element (SRE) is required for activation of the human Δ6D gene by SREBP-1. Moreover, the same SRE also mediates the suppression of the Δ6D gene by HUFA. PP induce fatty acid oxidation enzymes and desaturases in rodent liver. However, the induction of desaturases by PP is slower than the induction of oxidation enzymes. This delayed induction could be a compensatory response to the increased HUFA demand caused by peroxisome proliferation and induction of FA oxidation (Nakamura and Nara, 2002). In this study, the inhibition of Δ6D gene by HUFA was clearly shown. However, the delayed activation by PP was not found (results not presented here), which could explain that the sharp stimulation of the Δ6D gene in conditioned fish could be due to other mechanisms: at first, it is likely that SREBP-1a, which is normally high in dividing cells such as cell lines, is still expressed in conditioned larvae (a maintain of some primary features has been already observed in seabass larvae) resulting in a possible synergistic stimulation by SREBP-1a and SREBP-1c (the form expressed in differentiated cells including hepatocytes). Second, the affinity between SRE and SREBP could have been stimulated (by post-transcriptional events) in fish fed the HUFA deprived diet during larval stage. Finally, epigenetic modifications of the Δ6D gene could have occurred in conditioned fish. Pontoglio et al. (1997) identified hepatocyte nuclear factor 1 α (HNF1 α), which is a homeoprotein that is expressed in liver, kidney, pancreas and digestive tract. They showed that HNF1 α could activate transcription through the participation in the recruitment of the general transcription machinery to the promoter, or through the remodelling of chromatin structure and demethylation that would allow transcription factors to interact with their cognate *cis*-acting elements.

As some post-transcriptional regulation occurs, the Δ6D enzymatic activity may differ from the Δ6D gene expression. So, our results have to be confirmed by enzymatic activity measurements. The stimulation in Δ6D expression was not observed at the end of the experiment (d-211). This finding could be the consequence of an adaptation of the fish to the diet during the second period of the experiment d-181-d-211, revealing that the diet was not low enough in HUFA, and this was confirmed by the lack of breakdown in the growth curve. HUFA content in fish could be high enough to inhibit the Δ6D gene expression. The stimulation in Δ6D expression during the first period resulted from the diet change from a commercial feed (rich in HUFA, 2.7% EPA+DHA) to the experimental one (low level in HUFA, 0.5% EPA+DHA), suggesting that a significant expression of Δ6D is only obtained and maintained during extreme nutritional conditions.

Whole body DHA content at the end of the experiment was only affected by larval life history. The high DHA content measured in the fish at the beginning of the experiment (Fig. 5) was the consequence of feeding the rich-DHA commercial diet during the intermediate period. The differences observed in DHA content in PL as in TL at d-211 between the ex-22 and the ex-16 groups can be mainly explained by the higher relative growth of the smallest fish. The dilution of initial FA stores should affect the final FA content and this effect is growth dependant (Robin *et al.*, 2003). Dilution effects could also partly explain the differences in DHA in TL between the ex-LH and the ex-HH groups but not in PL content. The observed minor differences in PL content could partly result from larval nutritional conditioning. The higher DHA content in PL measured in the ex-LH groups than in the ex-HH groups is concomitant with the increase in mRNA Δ6D expression observed in the ex-LH juveniles. Increased Δ6D gene expression could lead to an increase in Δ6D enzymatic activity required for the first step of the bioconversion of 18 carbons into HUFA (20-22 carbons) and for bioconversion of EPA to DHA (Buzzi et al., 1997). The Δ6D enzyme uses 18:3n-3 (alpha-linolenic acid) as a substrate, and transforms it in 18:4n-3 (stearidonic acid) adding a double bond. This first step of desaturation allows the subsequent formation of EPA and DHA (Cho et al., 1999). Produced DHA should be primarily incorporated in PL. The total quantity of PL in fish is relatively low (near 0.7% WW). It was estimated in the present experiment that 1% increase in DHA in PL required a production of about 0.4-0.5 pmole.mg protein^{-1}. h^{-1} on whole fish basis. Such order of magnitude seems consistent with values measured in hepatocytes and enterocytes of sea bass fed on different diets (Mourente *et al.*, 2005). However, the validity of such comparison is difficult to assess. Other mechanisms, such as an improved capacity to incorporate DHA in PL, could also be involved. Compared to DHA, differences occurring for other FAs could be more related to weight increase than to a direct effect of larval conditioning. The high levels of DHA in PL versus TL, as well as the moderate decrease of DHA in PL of fish fed deficient diets are in accordance to the preferential incorporation of this FA in PL (Linares and Henderson, 1991). They are in the usual range in sea bass juveniles (Skalli and Robin, 2004) fed diets containing around 0.7% DM of n-3 HUFA. This study confirms that in this species, the minimal level of DHA in PL is about 20% of FAME (fatty acid methyl esters). Further studies are required to better understand the desaturation/elongation process, under more severe HUFA-deficient conditions, concomitantly measuring the specific enzymatic activity of the Δ6D enzyme.

Conclusion

While no significant effect of larval life history on juvenile growth performances in terms of survival and weight gain were observed in this study, an effect of nutritional conditioning on lipid metabolism was evidenced in juveniles fed a HUFA-deficient diet (0.5% DM n-3 HUFA) by (i) a positive modulation of the Δ6D transcription and (ii) by a higher capacity to regulate PL FAs when conditioned during the larval stage with a HUFA-deficient diet.

Acknowledgements

This work was supported by an IFREMER (French Research Institute for the exploitation of the Sea) and an INRA (National Institute of Agronomic Research) grants to the first author. We are grateful to N. Le Bayon, H. Le Delliou, M.M. Le Gall, J. Moriceau, P. Quazuguel and A. Severe for their technical assistance.

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Table 1. Formulation (g.100 g^{-1}) and chemical composition (% DM) of the experimental HUFA-deprived diet and the commercial diet.

[a] Sources: lupin without pellicle: Le Gouessant® aquaculture (Lamballe, France); fish meal LT 94: Norse (Fyllingsdalen, Norway); wheat amygluten 110: Chamtor Vitalor (Bazancourt, France); fish protein hydrolysate CPSP 90: Sopropêche (Boulogne sur mer, France); precooked starch: Prégéflo Roquette frères (Lestrem, France).

Ingredients [a]	Experimental diet	Commercial diet
Lupin without pellicle	50	
Fish meal LT 94	20	
Wheat amygluten 110	7	
Fish hydrolysate CPSP 90	8	
Vitamin mixture [b]	1	
Mineral mixture [c]	1	
Methionine	0.2	
Precooked starch	4.8	
Rapeseed oil	8	
Chemical composition		
Dry matter (%)	93.4	90.8
Crude protein (% DM)	52.1	55.2
Crude fat (% DM)	16.8	12.6
Ash (% DM)	6.5	12.3
n-3 HUFA (% DM)	0.48	2.68

[b] Vitamin mixture (g kg^{-1} vitamin mix): retinyl acetate, 1; cholecalciferol, 2.5; DL-α-tocopheryl acetate, 5; menadione, 1; thiamin-HCL, 0.1; riboflavin, 0.4; D-calcium panththenate, 2; pyridoxine-HCL, 0.3; cyanocobalamin, 1; niacin, 1; choline, 200; ascorbic acid (ascorbyl polyphosphate), 5; folic acid, 0.1; D-biotin, 1; meso-inositol, 30.

[c] Mineral mixture (g kg^{-1} mineral mix): KCL, 90; KI, 0.04; $CaHPO_4$ $2H_2O$, 500; NaCl, 40; $CuSO_4$ $5H_2O$, 3; $ZnSO_4$ $7H_2O$, 4; $CoSO_4$, 0.02; $FeSO_4$ $7H_2O$, 20; $MnSO_4$ H_2O, 3; $CaCo_3$, 215; MgOH, 124; Na_2SeO_3, 0.03; NaF, 1.

Table 2. Primer used for the Δ-6 desaturase gene expression analysis by RT-PCR.

Forward: 5'-3'	(GCCCTATCATCACCAACACC)
Reverse: 3'-5'	(ACAGCACAGGTAGCGAAGGT)

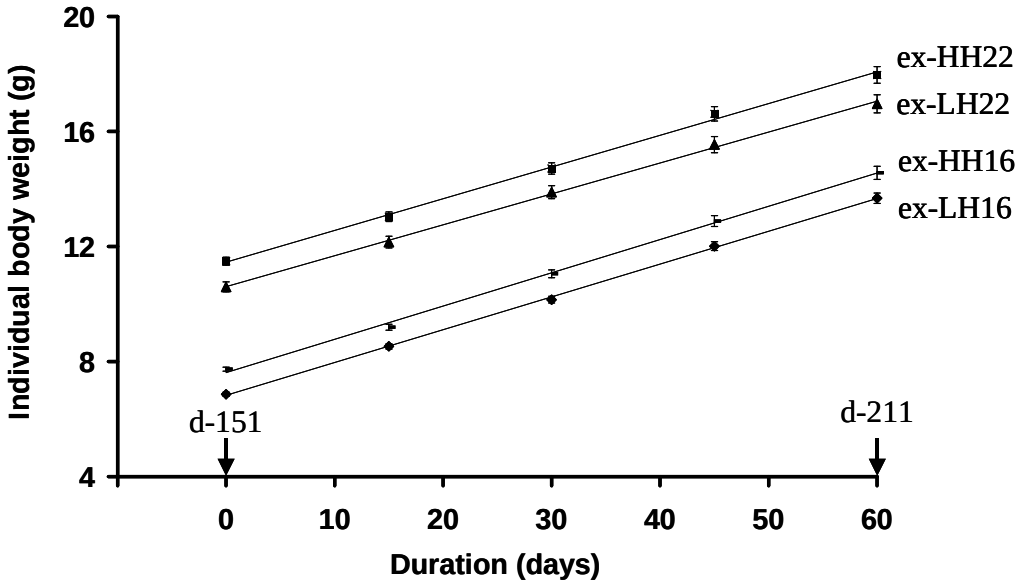

Fig.1: Mean body weight (± SE) increase over time (d-151 to d-211) for the 4 experimental sea bass groups. Equation of each regression curve was:
ex-HH22: $y = 0.12x + 11.30$; $R^2 = 0.9954$; n = 632
ex-LH22: $y = 0.11x + 10.45$; $R^2 = 0.9976$; n = 632
ex-HH16: $y = 0.12x + 7.45$; $R^2 = 0.9965$; n = 948
ex-LH16: $y = 0.12x + 6.66$; $R^2 = 0.9982$; n = 716

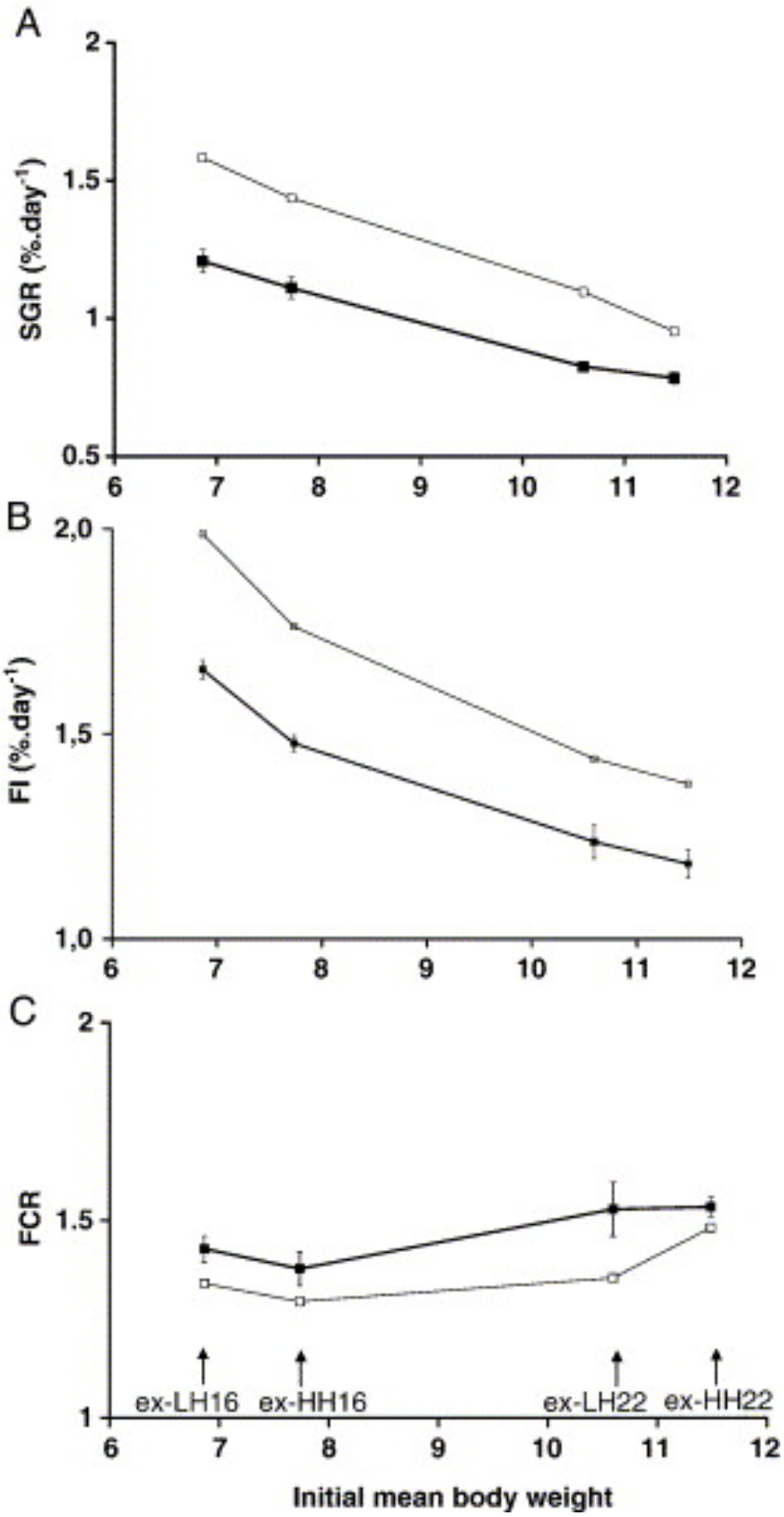

Fig.2: Influence of initial mean body weight on SGR, FI and FC (2A: SGR; 2B: FI and 2C: FC) in the 4 experimental groups fed the HUFA-deprived diet (full symbols ±SE) and in QR fed commercial diet (open symbols).

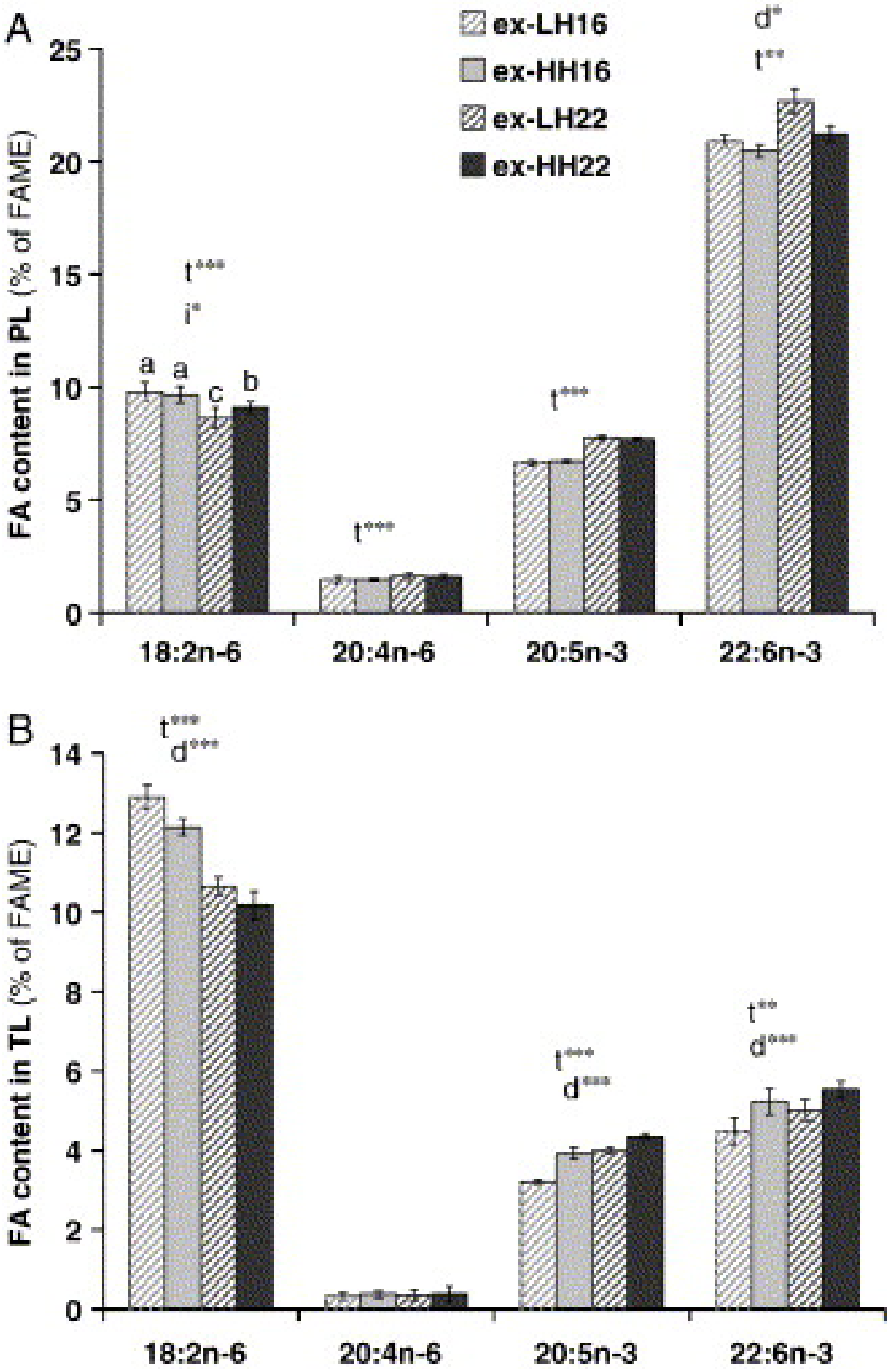

Fig.3: Fatty acid content (mean ± SE) in polar lipids, PL (Fig.A) and in total lipids, TL (Fig.B) at d-211. For each fatty acid represented, effect of temperature (t), diet (d) and interactions (i) are indicated: *P<0.05, **P<0.01 and ***P<0.001. (n = 4 replicates for each conditions).

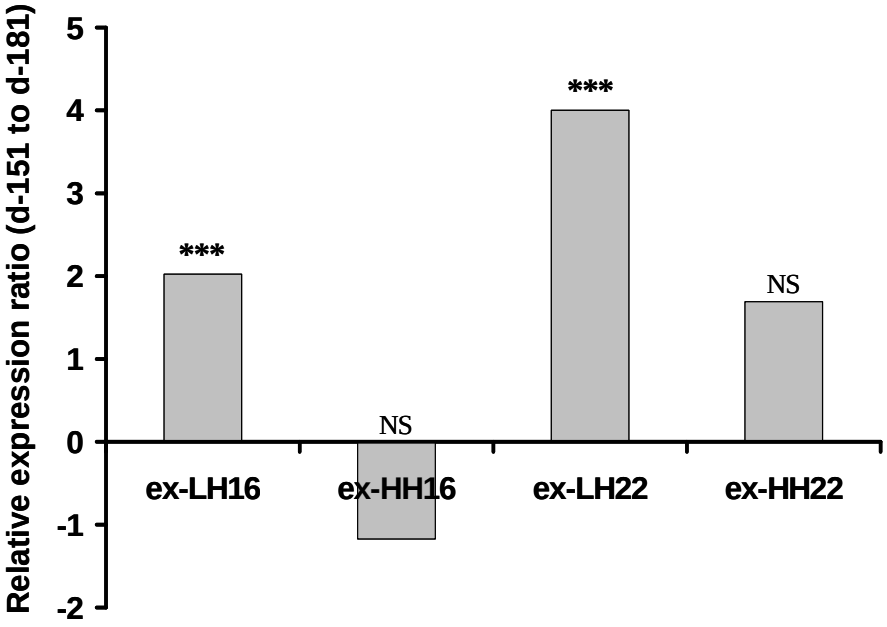

Fig.4: Delta-6 desaturase expression variation in the 4 experimental groups from d-151 to d-181, respect to the larval conditioning (n = 9 at d-151 and d-181 for each condition). *** denotes a significant difference (P<0.001), and NS a 'not significant difference' (P>0.05).

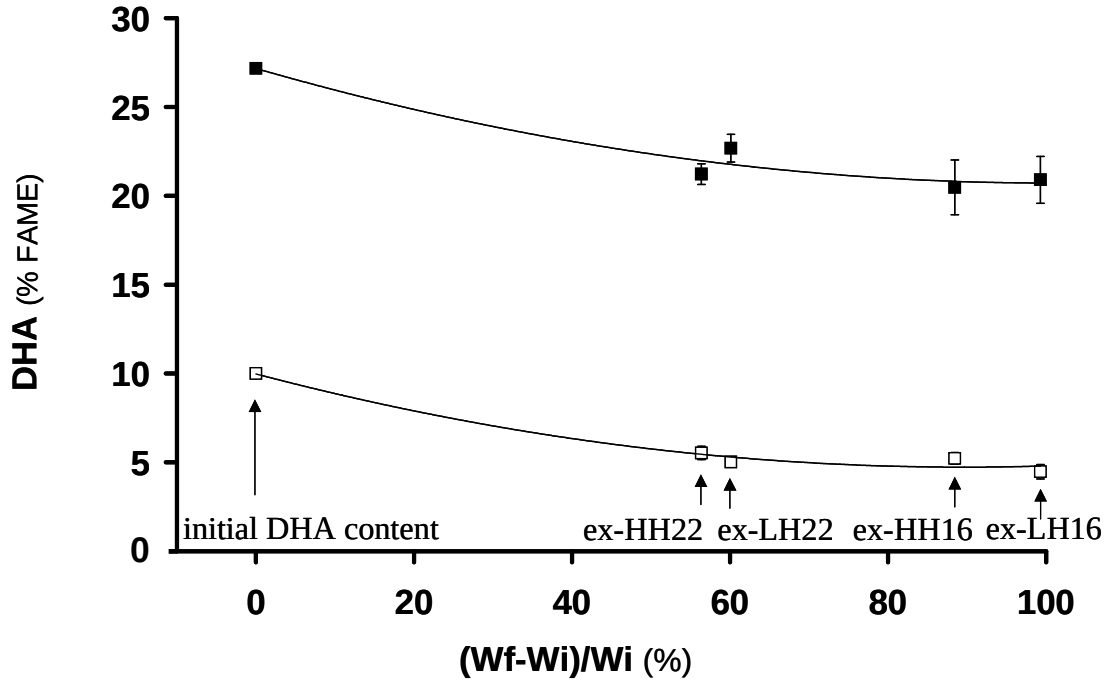

Fig.5: Influence of relative growth: (Wf-Wi)/Wi (where Wf= final weight and Wi = initial weight) on DHA content (mean ± SE) in PL (full symbols) and LT (open symbols) in the 4 experimental groups fed the HUFA-deprived diet. Values at X=0 correspond to the initial fish weight previously fed on a commercial diet (for X = 0, n = 3 and for others X values, n = 4 replicates of 8 fish per sample for the ex-16 groups and 6 fish for ex-22 groups). Equation of each polynomial curve was:
PL: $y = 0.0007x^2 - 0.117x + 9.9766$; $R^2 = 0.9785$
TL: $y = 0.0006x^2 - 0.128x + 27.169$; $R^2 = 0.9492$