

# Nutritional programming in sea bass

## Ontogenic effects of early feeding of sea bass (*Dicentrarchus labrax*) larvae with a range of dietary n-3 HUFA levels on the functioning of PUFA desaturation pathways

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### Abstract

Four replicated groups of sea bass larvae were fed XH (3.7% EPA+DHA), HH (1.7%), LH (0.7%) or XLH (0.5%) diets from d-6 to d-45 (XH<sub>I</sub>, HH<sub>I</sub>, LH<sub>I</sub>, XLH<sub>I</sub>; exp.1). After a subsequent one-month period feeding a commercial diet (2.7% EPA+DHA), the capacity of the four initial groups to adapt to an n-3 HUFA-Restricted diet (0.3% EPA+DHA; R-groups: XH<sub>2R</sub>, HH<sub>2R</sub>, LH<sub>2R</sub>, XLH<sub>2R</sub>) was tested for 35 days. Larval dietary treatments had no effect on larval and juvenile survival rates. Wet weight of d-45 larvae was higher in XH<sub>I</sub> and HH<sub>I</sub> (P<0.001), but the R-juvenile mass gains were similar in all treatments. Delta-6-desaturase ( $\Delta 6D$ ) mRNA level was higher in LH<sub>I</sub> and XLH<sub>I</sub> at d-45 (P<0.001), and higher in LH<sub>2R</sub> and XLH<sub>2R</sub>, with a significant increase at d-118. Concomitantly, PPAR  $\alpha$  and  $\beta$  mRNA levels were higher in XLH<sub>I</sub> at d-45, and PPAR  $\beta$  and  $\gamma$  mRNA levels were higher in the XLH<sub>2R</sub> at d-118, suggesting possible involvement of PPARs in stimulation of  $\Delta 6D$  expression, when drastic dietary larval conditioning occurred. The low DHA content in phospholipid (PL) of LH<sub>I</sub> and XLH<sub>I</sub> revealed an n-3-HUFA deficiency in these groups. Larval conditioning did not affect DHA content in PL of R-juveniles. This study showed (i) a persistent  $\Delta 6D$  mRNA

35 enhancement in juveniles pre-conditioned with an n-3 HUFA deficient larval diet, over the  
36 one-month intermediate period, and (ii) brought new findings suggesting the involvement of  
37 PPARs in the  $\Delta 6D$  mRNA level stimulation. However such nutritional conditioning had no  
38 significant effect on juvenile growth and lipid composition.

39

## 40 **Introduction**

41

42 Worldwide supplies of fish oils and meals have reached their sustainable limits, forcing  
43 industries to look for alternative lipid sources for use in marine fish diets <sup>(1)</sup>. As terrestrial  
44 animal products are prohibited, there is great interest in aquaculture to produce fish better able  
45 to utilise vegetable feedstuffs. Vegetable products are rich in 18 carbon fatty acids ( $C_{18}$  FA)  
46 but do not contain  $C_{20-22}$  n-3 highly unsaturated fatty acids (n-3 HUFA), such as  
47 ecosapentaenoic (EPA; 20:5n-3) and docosahexaenoic acids (DHA; 22:6n-3). These n-3  
48 HUFA are required in the diet to provide the essential fatty acids for marine fish, as marine  
49 fish have a low capacity to produce  $C_{20-22}$  HUFA from  $C_{18}$  FA precursors, such as  $\alpha$ -linolenic  
50 (18:3n-3) and linoleic (18:2n-6) acids <sup>(2,3)</sup>. Delta-6 desaturase ( $\Delta 6D$ ) is the rate-limiting  
51 enzyme catalysing the first reaction of n-3 HUFA synthesis from 18:3n-3 and 18:2n-6 <sup>(4)</sup>, but,  
52 as its activity is very low in marine fish <sup>(3,5)</sup>, it could also limit the use of vegetable products  
53 by marine fish.

54 One solution could be to apply metabolic programming using nutritional conditioning  
55 during early larval stages, as already shown in mammals <sup>(6)</sup>, in order to stimulate the FA  
56 desaturation pathways of n-3 HUFA synthesis in marine fish. We recently showed <sup>(7,8)</sup> that  
57 metabolism in sea bass (*Dicentrarchus labrax*) juveniles can be modulated by larval  
58 nutritional conditioning. The  $\Delta 6D$  mRNA level was enhanced in larvae fed a low n-3 HUFA  
59 diet (0.8% DM EPA+DHA), and this was retained in pre-conditioned juveniles fed an n-3  
60 HUFA-restricted diet (0.5% DM EPA+DHA). Moreover, a slightly, but significantly, higher  
61 DHA content in phospholipid (PL) in pre-conditioned juveniles was measured. However, the  
62 larval conditioning did not significantly affect growth performance of juveniles in terms of  
63 weight and survival rates, suggesting that larval nutritional stimulus was not sufficient to  
64 induce further long-term effects.

65 The aim of the present study was to determine the range of dietary n-3 HUFA content  
66 that would elicit effects on desaturation/elongation pathways for n-3 HUFA synthesis and  
67 whether the effect could be amplified. Thus, a large range of n-3 HUFA dietary content (0.5-

68 3.7% EPA+DHA) was used during the larval stage, followed by a severe n-3 HUFA-restricted  
69 diet (0.3% EPA+DHA) during the juvenile period.

70

## 71 **Materials and methods**

72

### 73 *Rearing conditions and experimental design*

74

#### 75 *Larval conditioning: Experiment 1*

76 Three days post-hatching sea bass (*Dicentrarchus labrax*) larvae were obtained from a  
77 commercial hatchery (Gravelines, France), and experiments were conducted at the Ifremer-  
78 Brest facility (Brest, France). Larvae were distributed in 20 conical fiberglass tanks (35 l;  
79 initial stocking density: 60 larvae l<sup>-1</sup>, *i.e.* 2500 larvae tank<sup>-1</sup>) and temperature was  
80 progressively increased from 13.5°C to 19°C within 2 days. All groups were fed  
81 microparticulate diets from mouth opening at day 6 (d-6) to d-45. Four experimental diets  
82 differing only in their n-3 HUFA content were tested: XH (3.7% EPA+DHA on a DM basis);  
83 HH (1.7%); LH (0.7%) and XLH (0.5%) (Table 1). The different n-3 HUFA contents were  
84 obtained by the incorporation of soybean oil in LH and XLH diets and by an inverse  
85 proportion gradient of soy lecithin and marine phospholipid. Four tanks were fed the HH or  
86 XH diets and six tanks were fed the LH or XLH diets. The four experimental conditions were  
87 XH1, HH1, LH1 and XLH1. The rearing conditions were as described previously <sup>(7)</sup>.

88

#### 89 *Juvenile period: Experiment 2*

90 The larval period was followed by an intermediate period of one month (d-46 to d-77),  
91 during which the four groups were separately held at 19°C and fed a commercial diet with  
92 2.7% EPA+DHA (DM basis), corresponding to the mean between the XH (3.7% EPA+DHA)  
93 and HH (1.7% EPA+DHA) diets. The four experimental groups were XH2, HH2, LH2 and  
94 XLH2. The d-77 juveniles of each group were anaesthetised (ethylene-glycol-  
95 monophenylether, 0.15‰) and selected fish were randomly distributed in 60 l square tanks  
96 (180 fish per tank). The fish were acclimatised to the experimental unit for 6 days (d-77-83) at  
97 19°C, and two experimental isolipidic and isoproteic diets differing in their n-3 HUFA  
98 content by the incorporation of either rapeseed oil or cod-liver oil (Table 2), were  
99 progressively introduced. Four replicate groups per initial condition were fed the experimental  
100 n-3 HUFA-Restricted diet (0.3% EPA+DHA); termed “R-groups” (XH2<sub>R</sub>, HH2<sub>R</sub>, LH2<sub>R</sub> and  
101 XLH2<sub>R</sub>). Two other replicated groups per initial condition were fed an n-3 HUFA-rich diet

102 (1.4% EPA+DHA, *i.e.* 2-fold higher than the 0.7% EPA+DHA requirement defined for sea  
103 bass juveniles <sup>(9)</sup>) and used as control “C-groups” (XH2<sub>C</sub>, HH2<sub>C</sub>, LH2<sub>C</sub> and XLH2<sub>C</sub>). The  
104 rearing conditions of juveniles were as described previously <sup>(8)</sup>. The experiment started when  
105 all groups were fed entirely on the HUFA-restricted or -rich diets (d-83, *i.e.* t0), and lasted  
106 until the final weights of all fish were increased at least two-fold (d-118; *i.e.* t35).

107

## 108 *Sampling procedures*

109

### 110 *Experiment 1*

111 For larval growth assessment and lipid composition, samplings were performed on 12 h  
112 fasted larvae at d-45, corresponding to the end of the larval period (when all enzymatic and  
113 molecular functions are established). For molecular analyses, intermediate samplings were  
114 also performed at d-10, d-17 and d-25.

115 Weight was monitored by sampling 30 larvae in four tanks per condition (120 larvae per  
116 condition; n=4). After a minimum preservation period of three weeks in 4% seawater  
117 formalin, larvae were individually weighed, pooled and dried for 24h at 105°C to estimate the  
118 dry weight of each group (n=4). Final biomass (mg.l<sup>-1</sup>) was the larvae mean wet weight per  
119 survival rate at d-45 (n=6 for XLH/ and LH/ and n=4 for XH/ and HH/). The survival rate  
120 was the ratio final/initial number of larvae in each tank, minus the number of larvae sampled  
121 (n=6 for XLH/ and LH/ and n=4 for XH/ and HH/).

122 Measurement of mRNA level of genes involved in digestive functions and lipid  
123 metabolism (delta-6 desaturase Δ6D, and peroxisome-proliferator activated receptors alpha  
124 PPAR α, beta PPAR β and gamma PPARγ) was performed on 100 mg of larvae at d-10 and d-  
125 17 and on about 300 mg of larvae at d-25 and d-45, in four tanks per condition (n=4). Larvae  
126 were conserved in TRIzol reagent (Invitrogen, Carlsbad, CA, USA) (1 ml for 100 mg of  
127 larvae) at -80°C pending analysis.

128 For lipid analysis, 50 pooled larvae from each of four tanks per condition (n=4) were  
129 weighed and conserved at -80°C pending analysis.

130

### 131 *Experiment 2*

132 Juveniles were anaesthetised before sampling. For all samplings, n=4 and n=2 for R-and  
133 C-groups, respectively. A HH2<sub>C</sub> tank was lost at d-90, inducing n=1 for this group at d-90, d-  
134 104 and d-118.

135 Growth was estimated by weighing individually 50 fish ( $\pm 0.1$  g) at d-83 (t0), d-90 (t7),  
136 d-104 (t21) and d-118 (t35) in all tanks. The survival rate was estimated as for larvae. The  
137 daily growth index (DGI; %) between t0 (d-83) and t35 (d-118) was calculated as follow:

$$138 \quad \text{DGI}_{\text{d-83-118}} = 100 \times (\sqrt[3]{w_{\text{d-83}}} - \sqrt[3]{w_{\text{d-118}}}) \text{ day}^{-1}$$

139 Measurement of mRNA level of genes involved in lipid metabolism ( $\Delta 6\text{D}$ , and PPAR  $\alpha$ ,  
140  $\beta$  and  $\gamma$ ) was performed on R-groups on 10 pooled fish from each of four tanks per condition  
141 (40 fish per condition) at d-83, d-90, d-107 and d-118. They were immediately frozen in  
142 liquid nitrogen and stored at  $-80^{\circ}\text{C}$  until assayed.

143 Lipid analyses were performed on C- and R-groups. Six pooled fish per tank were taken  
144 at d-83 and 10 pooled fish per tank were taken at d-118. They were individually weighed,  
145 frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  pending analysis.

146

#### 147 *Analytical methods*

148

#### 149 *Gene expression*

150 Expression of  $\Delta 6\text{D}$ , and PPAR  $\alpha$ , PPAR  $\beta$  and PPAR  $\gamma$  genes was performed on whole  
151 body for larvae and on liver for juveniles. Dissections of frozen juveniles were conducted on a  
152 glass plate maintained at  $0^{\circ}\text{C}$ . The whole liver was isolated and the gall-bladder removed  
153 because bile can destroy RNAs. Gene expression measurements of each sample were  
154 performed on 200 mg of homogenised pooled livers (Polytron® PT 2100 Bioblock®). Total  
155 RNA was extracted from total larvae and livers using TRIzol reagent (Invitrogen, Carlsbad,  
156 CA, USA). cDNAs were obtained in duplicate from total RNA (1  $\mu\text{g}$ ) using Quantitect  
157 Reverse Transcription® kit with integrated removal of genomic DNA contamination  
158 (QIAGEN® GmbH, Hilden, Germany). Real-time PCR was performed using the iCycler  
159 iQTM® (Bio-Rad® Laboratories Inc., Hercules, CA, USA) as described in our previous  
160 studies <sup>(7,8)</sup>. The specificity of forward and reverse primers for each gene was checked by  
161 sequencing the amplicon (Eurogentec, Labège, France). Primers for  $\Delta 6\text{D}$ , PPAR  $\alpha$  and PPAR  
162  $\beta$  were as described previously <sup>(7)</sup>. Those for PPAR  $\gamma$  were 5'-3':  
163 CAGATCTGAGGGCTCTGTCC and 3'-5': CCTGGGTGGGTATCTGCTTA. Real-time  
164 PCR efficiencies were determined for each gene from the given slopes in Bio-Rad® software  
165 (iCycler iQ™ Real-Time Detection System Software, Bio-Rad® Laboratories Inc., Hercules,  
166 CA, USA), according to the equation 1:

167 
$$E=10^{[-1/\text{slope}]}$$

168 To determine the relative quantity of target gene-specific transcripts present in the different  
169 samples, expression ratios (R) were calculated according to the following formula (2):

170 
$$\text{Ratio}=[(E_{\text{gene}})^{\Delta \text{CT target gene (mean control-mean sample)}}]/[(E_{\text{EF1}})^{\Delta \text{CT EF1 (mean control-mean sample)}}]$$

171 where “E” is the PCR efficiency and “mean sample” corresponds to triplicate average. The  
172 HH1 and HH2 samples were used as the standard group for larval and juvenile experiments,  
173 respectively, because they are close to the rearing condition in fish farming. Elongation factor  
174 1  $\alpha$  (EF1  $\alpha$ ) was used as the reference gene <sup>(10)</sup> as its expression is constant during activation  
175 and proliferation of cells <sup>(11)</sup>.

176

### 177 *Fatty acid composition*

178 Whole frozen larvae were homogenised at 0°C using a Polytron® (PT 2100 Bioblock®,  
179 Illkirch, France), while whole frozen juvenile were homogenised rapidly with a Hobart®  
180 mixer (Sydney, Australia) in order to keep a low temperature and then, more accurately using  
181 a Polytron® (PT 2100 Bioblock®, Illkirch, France). Lipid analyses were performed on a  
182 representative portion (~1 g and ~5g for larvae and juvenile samples respectively) and ~3 g  
183 were taken for dry weight measurement (105°C; 24h). Assays were conducted on one larval  
184 sample, while for juveniles they were performed on duplicates at d-83 and at d-118.

185 Extraction of total lipid (TL), separation of neutral (NL) and polar lipid (PL), preparation  
186 of fatty acid methyl esters (FAME) and separation of FAME were performed on larvae and  
187 juveniles as described in our previous study <sup>(7,8)</sup>. Each chromatogram was visually controlled  
188 on the computer using an amplification of the baseline in order to check the peak shape and  
189 quality of integration by the computer program. Internal standard (tricosanoic acid 23:0) was  
190 used quantify FAME in TL and NL on a fish fresh matter basis, and was added to a weighed  
191 known quantity of larvae before the TL extraction, while it was added before the TL and PL  
192 FAME extraction for juvenile lipid analysis. The results of individual FA compositions were  
193 expressed as percent of total identified FAME.

194 Chemical analyses of feed were performed in duplicate for each sample according to  
195 AOAC <sup>(12)</sup> methods.

196

### 197 *Statistical analysis*

198

199 The data are presented as mean  $\pm$  S.E. of the replicate groups. Before applying statistical  
200 tests using Statistica® (Tulsa, Oklahoma, USA), percentage data were transformed by arcsine

201 square root, and data for body weight, biomass and relative gene expression ratio were  
202 transformed by Ln. Effect of diet on growth performances, mRNA level for each sampling  
203 date and lipid composition was tested on means per tank using a one-way ANOVA, after  
204 control of equality of variances using Levene test. Effects of diet and age of fish on mRNA  
205 level were tested on means per tank using two-way ANOVA, after control of equality of  
206 variances using Chi-deux test. Effect of larval nutritional conditioning on mass gain of R-  
207 groups of juveniles, as well as between R-and C-groups was tested comparing curve slopes  
208 between t0 and t7; t7 and t21 and t21 and t35 by a one-way ANOVA. The Newman-Keuls  
209 multiple-range test was used to compare means in case of a significant effect ( $P<0.05$ ).

210

## 211 **Results**

212

### 213 *Experiment 1*

214

#### 215 *Growth performances*

216 Diet did not significantly influence larval survival rate ( $46.0\pm 2.3\%$ ), while the mean wet  
217 weight was more than 25% higher in XH/ and HH/ groups than in LH/ and XLH/ groups  
218 (Fig.1A;  $P<0.001$ ). The mean final biomass of XLH/ groups ( $892\pm 108\text{ mg.l}^{-1}$ ) was around  
219 34% lower than that of XH/ and HH/ ( $1367\pm 47\text{ mg.l}^{-1}$ ;  $P<0.01$ ), while biomass measured in  
220 LH/ groups ( $1093\pm 93\text{ mg.l}^{-1}$ ) was not significantly different from the others (Fig.1B).

221

#### 222 *Gene expression*

223 At d-10, the  $\Delta 6D$  mRNA level was similar in all groups (Fig.2A;  $1.0\pm 0.2$ ). At d-17, LH/  
224 and XLH/ groups exhibited higher values than XH/ ( $P<0.05$ ) but were similar to HH/. The  
225 difference between groups fed a low-HUFA diet (XLH/, LH/) and those fed a rich-HUFA  
226 diet (XH/, HH/) increased with time, and mean  $\Delta 6D$  mRNA level measured in XLH/ and  
227 LH/ groups was higher than that of XH/ and HH/ groups at d-45 ( $P<0.01$ ).

228 One-way ANOVA analysis revealed that PPAR  $\alpha$ ,  $\beta$  and  $\gamma$  mRNA levels were not  
229 affected by diet from d-10 to d-25 (Fig.3A, 4A, 5A). At d-45, PPAR  $\alpha$  and  $\beta$  mRNA levels  
230 were higher in XLH/ groups than in others ( $P<0.05$ ), while PPAR  $\gamma$  mRNA level was higher  
231 in XLH/ groups than that measured in LH/ larvae ( $P<0.05$ ) but similar to that measured in  
232 XH/ and HH/ groups.

233

#### 234 *Lipid analysis*

235 TL content in d-45 larvae was low (between 2 and 3% wet weight WW) and there were  
236 no significant differences between treatments (Table 3). Differences in NL composition of  
237 larvae at d-45 reflected those of the diets (Tables 1, 3). However, 18:3n-6 was significantly  
238 higher in NL of XLH/ and LH/ larvae than in NL of XH/ and HH/ larvae ( $P<0.01$ )  
239 independent of 18:3n-6 dietary content. HUFA: AA (arachidonic acid 20:4n-6), EPA and  
240 DHA contents were high in PL of d-45 larvae, and increased from XLH/ to XH/, according  
241 to diet ( $P<0.05$ ). The 18:2n-6 and 18:3n-3 contents were lower in PL of larvae than in diets,  
242 and decreased from XLH/ to XH/, according to diet ( $P<0.001$ ). Other PUFA were low and  
243 not directly related to diet composition: 18:3n-6 and 20:3n-6 were significantly higher in PL  
244 of LH/ and XLH/ than in PL of XH/ and HH/ ( $P<0.001$  and  $P<0.05$  respectively) and  
245 20:2n-6 was lower in PL of XH/ than in PL of other groups ( $P<0.001$ ). The other  
246 intermediates in n-3 FA synthesis (18:4; 20:3, 20:4) were very low (0.12%; 0.06% and 0.20%  
247 of FAME, respectively) and their content was not different between groups (not presented in  
248 Table 3).

249 The PL content in d-45 larvae represented a major proportion of TL ranging from 41% to  
250 47% with a significantly higher value in XH/ fish than in LH/ and XLH/ groups ( $P<0.05$ ).  
251 Accordingly, TL FA profiles of larvae (not presented here) showed intermediate percentages  
252 between those obtained in NL and PL.

253

## 254 *Experiment 2*

255

### 256 *Growth performances*

257 Juvenile survival rate ( $98.3\pm 0.25\%$ ) was not affected by larval nutritional conditioning.  
258 There was no significant difference in weight increase from d-83 (t0) to d-118 (t35) in the  
259 four R-groups ( $2.2\pm 0.02$  g). The mass gain was similar between R-groups (Fig.6) and C-  
260 groups (not presented) from t0 to t7 (NS differences in curves slopes). From t7 to t21, mass  
261 increase was significantly higher in R-groups than in C-groups ( $P<0.01$ ) and significantly  
262 lower from t21 to t35 ( $P<0.01$ ). D-83 to d-118 daily growth index was not significantly  
263 different ( $P=0.075$ ) between R- ( $1.13\pm 0.02\%$ ) and C-groups ( $1.19\pm 0.00\%$ ).

264

### 265 *Gene expression*

266 The mean  $\Delta 6D$  mRNA level was 2-fold higher at d-118 than any time-point earlier  
267 (Fig.2B; two-way ANOVA;  $P<0.001$ ). One-way ANOVA performed at each sampling date  
268 indicated that  $\Delta 6D$  mRNA level was significantly higher in XLH<sub>2R</sub> and LH<sub>2R</sub> than in XH<sub>2R</sub>

269 groups at d-83 ( $P<0.05$ ), while XLH2<sub>R</sub> and HH2<sub>R</sub> showed similar values. At d-90, XLH2<sub>R</sub> and  
270 LH2<sub>R</sub> showed ~2-fold higher values than XH2<sub>R</sub> and HH2<sub>R</sub> ( $P<0.05$ ) and at d-107, XLH2<sub>R</sub>,  
271 LH2<sub>R</sub> and XH2<sub>R</sub> showed higher values than in HH2<sub>R</sub> ( $P<0.05$ ). At d-118, the  $\Delta 6D$  mRNA  
272 level was lowest in XH2<sub>R</sub> groups ( $1.4\pm 0.2$ ) and highest in XLH2<sub>R</sub> and LH2<sub>R</sub> groups ( $4.7\pm 0.5$   
273 and  $5.6\pm 0.6$  respectively;  $P<0.001$ ).

274 The mean PPAR  $\alpha$  and  $\beta$  mRNA levels were globally higher at the end of exp.2 (d-118)  
275 than any previous time-point (Fig.3B and 4B; two-way ANOVA;  $P<0.01$ ). Using one-way  
276 ANOVA, PPAR  $\alpha$  and  $\beta$  mRNA levels were similar in all R-groups at d-83 and d-90. At d-  
277 107, PPAR  $\alpha$  mRNA level was about 3-fold lower in XH2<sub>R</sub> than in other groups ( $P<0.01$ ),  
278 while PPAR  $\beta$  mRNA levels were similar in all groups. At d-118, PPAR  $\alpha$  mRNA level was  
279 similar in XH2<sub>R</sub> and LH2<sub>R</sub> groups, and about 50% lower than in HH2<sub>R</sub> and XLH2<sub>R</sub> groups  
280 ( $P<0.05$ ). In comparison, PPAR  $\beta$  was significantly higher in XLH2<sub>R</sub> groups than in other  
281 groups ( $P<0.01$ ). PPAR  $\gamma$  mRNA level was significantly lower at d-107 than at other  
282 sampling periods (Fig. 5B; two-way ANOVA;  $P<0.001$ ). At d-83, it was more than 3-fold  
283 higher in LH2<sub>R</sub> groups than in others ( $3.4\pm 0.6$ ;  $P<0.05$ ), while non-significant differences  
284 occurred between groups at d-90 and d-107. At d-118, XLH2<sub>R</sub> groups showed a significantly  
285 higher mRNA level than XH2<sub>R</sub> and HH2<sub>R</sub> ( $P<0.01$ ).

286

### 287 *Lipid analysis*

288 The mean whole body TL content of R-groups was not significantly different between  
289 groups during the course of the experiment (Table 4). It was  $4.4\pm 0.4$  % WW at d-83 and  
290  $9.2\pm 0.2\%$  at d-118. The PL content represented a higher proportion of TL at d-83 than at d-  
291 118 ( $29.5\pm 1.1$  vs.  $13.4\pm 0.9$  % TL). At d-83 (*i.e.* after one-month feeding the commercial diet),  
292 the influence of diets observed during larval stage disappeared, FA composition was very  
293 similar in all groups, except for DHA, which was higher in NL of XH2<sub>R</sub> groups than in others  
294 ( $12.4\pm 0.2$  vs.  $11.5\pm 0.0$  % FAME;  $P<0.05$ ). At d-118, the DHA, EPA, AA, 18:3n-6 and  
295 saturated fatty acid contents in NL of R-groups were higher than in the R-diet ( $P<0.05$ ), while  
296 18:2n-6, 18:3n-3, MUFA and PUFA levels were lower (Tables 4, 2). DHA, EPA and 20:3n-3  
297 were higher in NL of XH2<sub>R</sub> groups than in others at d-118 ( $P<0.01$  and  $P<0.05$  respectively).  
298 The 22:5n-3 content in NL of XH2<sub>R</sub> groups was higher than in LH2<sub>R</sub> and XLH2<sub>R</sub> ( $P<0.05$ ).  
299 Other FAs in NL were not significantly different within R-groups. From d-83 to d-118, 18:2n-  
300 6, 18:3n-3 and MUFA content in NL increased by 45%, 75% and 41% respectively, while  
301 other FA, including DHA, EPA and AA decreased ( $11.9\pm 0.2$  vs.  $2.4\pm 0.1$  % FAME for DHA).  
302 The 18:3n-6, AA, EPA and DHA contents were higher in PL of d-118-juveniles than in their

303 R-diet, while the contrary was observed for 18:2n-6 and 18:3n-3. From d-83 to d-118, 18:2n-  
304 6, 18:3n-6, 20:2n-6, 20:3n-6, 18:3n-3 and MUFA content in PL increased, while others FA,  
305 including DHA, EPA and AA decreased. FA content in PL was not significantly different  
306 within R-groups at d-83, as well as at d-118, except for 20:4n-3, higher in LH2<sub>R</sub> groups than  
307 in others (P<0.05). The AA, EPA, DHA, saturated FA and PUFA were noticeably higher in  
308 PL than in NL at d-83 and d-118.

309 The fatty acid content of C-groups was related to C-diet (not detailed here). Their HUFA  
310 content was clearly higher than in R-groups (not detailed here). The AA, EPA and DHA  
311 contents in PL of C-groups were 1.9±0.0, 9.8±0.2 and 24.2±1.3 % FAME respectively. The  
312 low number of replicates did not allow a statistical evaluation within C-groups.

313

## 314 **Discussion**

315

316 The aim of this study was to elucidate whether the stimulation of  
317 desaturation/elongation pathways for n-3 HUFA synthesis in juveniles induced by a larval  
318 nutritional conditioning shown in a previous study<sup>(8)</sup> can be amplified using a large range of  
319 n-3 HUFA content (0.5-3.7% EPA+DHA) in the larval diet and a severe n-3 HUFA-restricted  
320 diet (0.3% EPA+DHA; R-diet) during the juvenile period.

321 As encountered in other studies<sup>(7, 13)</sup>, diet composition had no significant effect on larval  
322 survival rates, while very low dietary n-3 HUFA content (XLH 0.5% EPA+DHA) led to  
323 decreased larval mass gain. Survival rates obtained were in agreement with a previous study  
324<sup>(13)</sup>, in which sea bass larvae reared at 19°C and fed a diet similar to HH/ had a survival rate  
325 of 48% at d-38. The effect of high dietary HUFA content on mass gain could be the  
326 consequence of an elevated n-3-HUFA requirement for high cellular turn-over during the  
327 larval stage<sup>(14)</sup>. Larval mean weights obtained in this study were high at d-45, and the values  
328 obtained in HH/ groups was much higher than found previously<sup>(13)</sup> in sea bass larvae reared  
329 in similar conditions. This could indicate the initial larvae were of high quality. As previously  
330 observed<sup>(8)</sup>, larval conditioning did not affect growth performance of sea bass juveniles fed  
331 the R-diet, despite large differences in juvenile initial weight at the onset of the experiment 2,  
332 as a result of the different n-3 HUFA contents of the larval diets. The weight increase of R-  
333 juveniles was good in all groups, as it more than doubled in 35 days, and was not significantly  
334 different from that observed in C-groups during the first week of the experiment. However,  
335 the growth of R-groups seemed to be limited during the last period of the experiment. This

336 was in accordance with a significant growth retardation of sea bass juveniles fed different  
337 HUFA dietary contents for 7 weeks<sup>(9)</sup>.

338 As observed earlier<sup>(7,8)</sup>, the level of  $\Delta 6D$  mRNA was significantly higher in larvae fed  
339 a restricted n-3 HUFA diet during the larval stage (0.5 or 0.7% EPA+DHA), and in juveniles  
340 fed a low HUFA diet (0.3% EPA+DHA), following a transient feeding on a HUFA diet. This  
341 revealed that (i)  $\Delta 6D$  transcription could be modulated by the n-3 HUFA content of the diet,  
342 as observed in seabream<sup>(15)</sup> and, that (ii) conditioned juveniles were better than unconditioned  
343 fish in better developing desaturation processes in order to adapt to a low dietary HUFA  
344 content. The mRNA expression data were supported by the significant increase in 18:3n-6  
345 measured in PL, as it is the  $\Delta 6D$  desaturation product of 18:2n-6, and could not have been  
346 obtained through the diet. These findings indicate that the increase in the level of  $\Delta 6D$  mRNA  
347 likely led to an increase in  $\Delta 6D$  enzymatic activity required for the first step of the  
348 bioconversion of 18 carbon FA to HUFA (20-22 carbons), and for the conversion of EPA to  
349 DHA<sup>(16)</sup>. Contrary to our previous study<sup>(8)</sup>, the present results showed a persistence of  $\Delta 6D$   
350 mRNA level in juveniles 30 days after feeding of the larval diets ceased, and beyond the  
351 intermediate period on a high HUFA diet. It may be a consequence of (i) the more restricted  
352 HUFA contents in the larval (0.5 and 0.7% EPA+DHA vs. 0.8%) and juvenile (0.3% vs.  
353 0.5%) diets used, (ii) from the younger fish used (d-83 vs. d-151 at the beginning of  
354 experiment 2), or (iii) from a shorter acclimation period (30 days vs. 90 days).

355 Our results demonstrated that PPAR  $\alpha$  and PPAR  $\beta$  genes, which are involved in FA  
356 catabolism and keratinocyte differentiation, showed (i) a higher mRNA level in d-45 larvae  
357 fed the lowest dietary n-3 HUFA content (0.5% EPA+DHA) and, (ii) this was maintained in  
358 d-118 juveniles in the case of PPAR  $\beta$ . These results were in concordance with the higher  
359  $\Delta 6D$  mRNA level measured in these groups at the same times. According to several studies  
360 conducted in mammals<sup>(17)</sup>, PPARs are involved, along with sterol regulatory element binding  
361 protein-1 (SREBP-1a and SREBP-1c), in the control of the  $\Delta 6D$  gene transcription. SREBP-1  
362 binds to sterol regulatory elements (SREs), and mediates the suppression of the  $\Delta 6D$  gene by  
363 HUFA. In the present study, the concomitant increase of PPARs and  $\Delta 6D$  mRNA levels  
364 suggested that PPARs could be partly involved in modulating  $\Delta 6D$  gene expression in larval  
365 and juvenile sea bass. PPAR  $\gamma$  is involved in adipocyte differentiation and induction of  
366 lipogenic enzymes and, although its mRNA level was not significantly higher in larvae fed an  
367 n-3 HUFA-deprived diet, it was significantly higher in d-118 juveniles pre-conditioned with  
368 the lowest n-3 HUFA diet during the larval stage. This suggested that PPAR  $\gamma$  could also have  
369 a role in the stimulation of the  $\Delta 6D$  gene expression observed at the same time. The

370 stimulation of PPAR mRNA level was not significantly higher in juveniles pre-conditioned  
371 with the LH diet (0.7% HUFA dietary content), in spite of the significantly higher  $\Delta 6D$   
372 mRNA level measured in these groups. We could hypothesise that the  $\Delta 6D$  gene could  
373 possibly be stimulated by PPARs when drastic nutritional conditions occurred, and that above  
374 a threshold, other mechanisms like those observed in mammals may be implicated, such as  
375 SREBP-1. This hypothesis is in concordance with previous results <sup>(8)</sup>, which did not reveal  
376 any significant stimulation of PPARs, using a conditioning larval diet containing 0.8%  
377 EPA+DHA, while a higher  $\Delta 6D$  mRNA level was observed.

378 As the increase in PPAR and  $\Delta 6D$  mRNA levels observed in larvae fed a low HUFA  
379 diet were retained in juveniles fed a low HUFA diet, this indicated that (i) pre-conditioned  
380 fish were able to develop adaptation to low dietary HUFA content during juvenile period and  
381 that (ii) this adaptation could be the consequence of nutritional programming occurring during  
382 larval stage. Several existing biological mechanisms described in mammals could explain the  
383 “memory” of metabolic effects of early nutritional environments in juveniles <sup>(18)</sup>: Induced  
384 variations in organ structure, alterations in cell number, clonal selection, metabolic  
385 differentiation, hepatocyte polyploidisation and epigenetic modifications. In this study,  
386 memory of metabolic process in juveniles could be due to epigenetic modifications of the  
387  $\Delta 6D$  and PPARs genes. Epigenetic modifications are modifications of DNA and covalent  
388 modifications of histones, which condition the accessibility of chromatin to transcription  
389 factors, facilitating the recognition of genes to be expressed or silenced, transiently or  
390 permanently, by these factors <sup>(18)</sup>. The hepatocyte nuclear factor 1  $\alpha$  (HNF1  $\alpha$ ) has been  
391 identified as a homeoprotein expressed in liver, kidney, pancreas and digestive tract that  
392 could activate transcription through participation in the recruitment of the general  
393 transcription machinery to the promoter, or through the remodelling of chromatin structure  
394 and demethylation that would allow transcription factors to interact with their cognate *cis*-  
395 acting elements <sup>(19)</sup>.

396 The relatively high level of n-3 HUFA measured in PL vs. NL of larvae and juveniles  
397 was in agreement with the preferential incorporation of these FA in PL contributing to the  
398 maintenance of PL quality <sup>(20)</sup>. Although AA, EPA and DHA were selectively incorporated in  
399 PL of larvae, low values were observed in PL of fish fed low n-3 HUFA diet (LH/ and  
400 XLH/), revealing an n-3 deficiency in these groups. Even though growth was similar in LH/  
401 and XLH/ larvae, HUFA content in PL was different within these groups, in accordance with  
402 values previously observed in d-45 sea bass larvae fed a diet with similar EPA+DHA content  
403 <sup>(7)</sup>. The XH/ groups showed an exceptionally high DHA content in both PL and NL in d-45

404 larvae, which has rarely been observed in aquaculture, except in larvae fed on natural  
405 plankton or on rotifers enriched with DHA <sup>(21, 22)</sup>. The AA deficiency observed in larvae fed  
406 the LHI and XLHI diet, while its precursor 18:2n-6 increased in these groups, can be  
407 explained by low activity of  $\Delta 5D$  in these groups. This hypothesis was in accordance with the  
408 very low enzymatic activity of  $\Delta 5D$  compared to that of  $\Delta 6D$  measured in seabream *Sparus*  
409 *aurata* <sup>(23)</sup>. That 18:4n-3 was not increased could be due to the higher concentration of this  
410 fatty acid in larvae combined with the low concentration of its precursor (18:3n-3) in the diets  
411 used, as shown in microsomes of dogs and rats <sup>(24)</sup>. Moreover, as the level of 18:2n-6 is ten-  
412 fold higher than 18:3n-3 in the diets, its bioconversion could be stimulated in larvae even  
413 although  $\Delta 6D$  usually shows higher affinity with n-3 fatty acids than with n-6 fatty acids <sup>(25)</sup>.  
414 This suggested that production of 18:4n-3 could exist in n-3 HUFA-deprived larvae, even if it  
415 is not observable with the techniques used. Thus, the high level of n-6 fatty acids in the diet  
416 may mask effects on the n-3 HUFA synthesis pathway.

417 D-83 R-juveniles showed a similar composition in NL, while at d-118, several n-3 HUFA,  
418 including EPA and DHA, were present at a higher level in NL of XH2<sub>R</sub> juveniles than in  
419 others. This indicated that differences observed at d-118 could be the consequence of the  
420 growth dependent-dilution effect of initial (d-83) FA stores in the smaller fish <sup>(26)</sup>. The DHA  
421 content in PL of R-juveniles at d-118 (about 17%; P<0.001) was intermediate between the  
422 DHA content of n-3 deprived LHI and sufficient HH1 larvae, and significantly lower than in  
423 C-groups (about 24%), and d-83 juveniles (about 27%). This was in agreement with a  
424 previous study <sup>(9)</sup>, which showed that sea bass juveniles fed at or above requirement had a  
425 minimal DHA content in PL of around 20% of total FA. The FA content in PL of d-118  
426 juveniles remained similar in all groups, except for 20:4n-3, which was at a higher level in  
427 XH2<sub>R</sub> groups than in others. In a previous experiment <sup>(8)</sup>, a slightly higher DHA content in PL  
428 was found in juveniles conditioned with a n-3 HUFA deprived diet during the larval stage  
429 than in others, suggesting an enhanced capacity to adapt to a restricted-HUFA diet. A similar  
430 result was not obtained in the present study, which could be the consequence of technical  
431 differences between the two experiments, or to biological mechanisms. Irrespective, the two  
432 studies showed that the observed stimulation of  $\Delta 6D$  mRNA was not linked to an increase in  
433 PL n-3 HUFA content, and this could be due to the very low rate of desaturation already  
434 described for European sea bass, even when up-regulated by diet <sup>(27)</sup>.

435

## 436 **Conclusion**

437

438 This study demonstrated an amplified stimulation of  $\Delta 6D$  mRNA induced by dietary n-3  
439 HUFA deficiency in juveniles pre-conditioned with a low dietary n-3 HUFA content during  
440 the larval stage, and persisting in young juveniles. However, this did not have a noticeable  
441 influence on FA composition and growth performances in juveniles challenged with a HUFA  
442 restricted diet. Our results also suggested the involvement of PPARs in the regulation of  $\Delta 6D$   
443 gene expression. Further studies concerning enzymatic activities of  $\Delta 6D$  and PPARs gene  
444 regulation are required to further investigate and understand the metabolic pathways for  
445 HUFA synthesis in marine fish.

446

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448

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M. Vagner



J.H. Robin



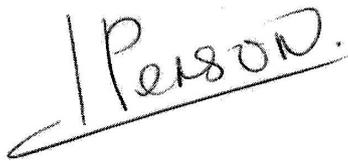
J.L. Zambonino Infante



D. Tocher



J. Person-Le Ruyet



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- 530

Fig.1 ; Vagner et al.

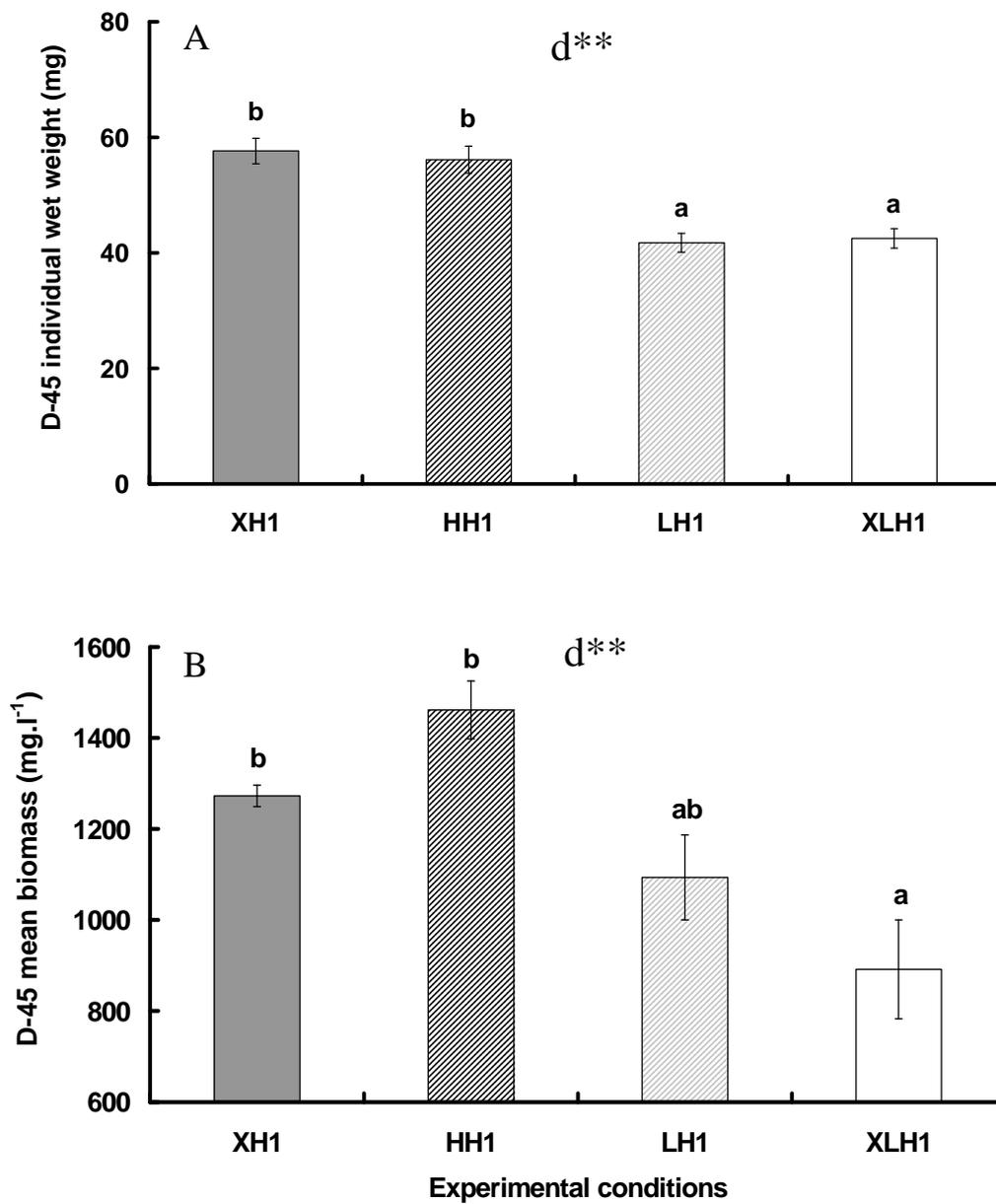


Fig.1. (A) D-45 mean larval wet weight (n=4) and (B) biomass (n=4 for XH1 and HH1 groups and n=6 for XLH1 and LH1 groups) at each experimental condition. Values are means  $\pm$  SE and statistical significance of diet (d) is indicated (\*\* P<0.01). Values not sharing a common letter are significantly different.

Fig.2 ; Vagner et al.

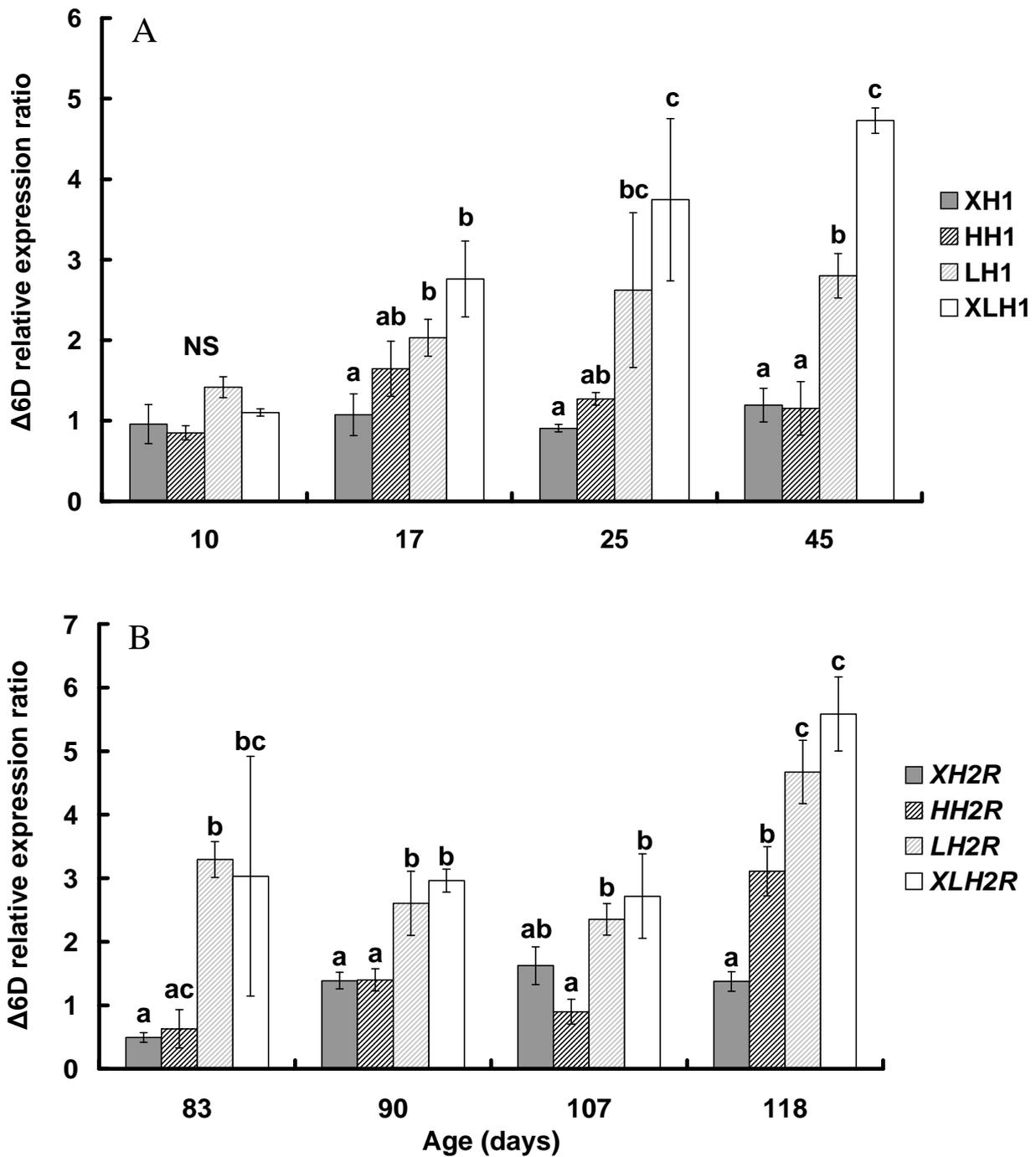


Fig.2. Mean  $\Delta 6$  desaturase gene expression ratio ( $\pm$  SE) relative to HH1 (A) and HH2<sub>R</sub> groups (B) according to time in larval (A) and juvenile (B) experiment and for each experimental condition (n=4). <sup>a, b, c</sup> differing letters denote significant difference for each date (P<0.05).

Fig.3 ; Vagner et al.

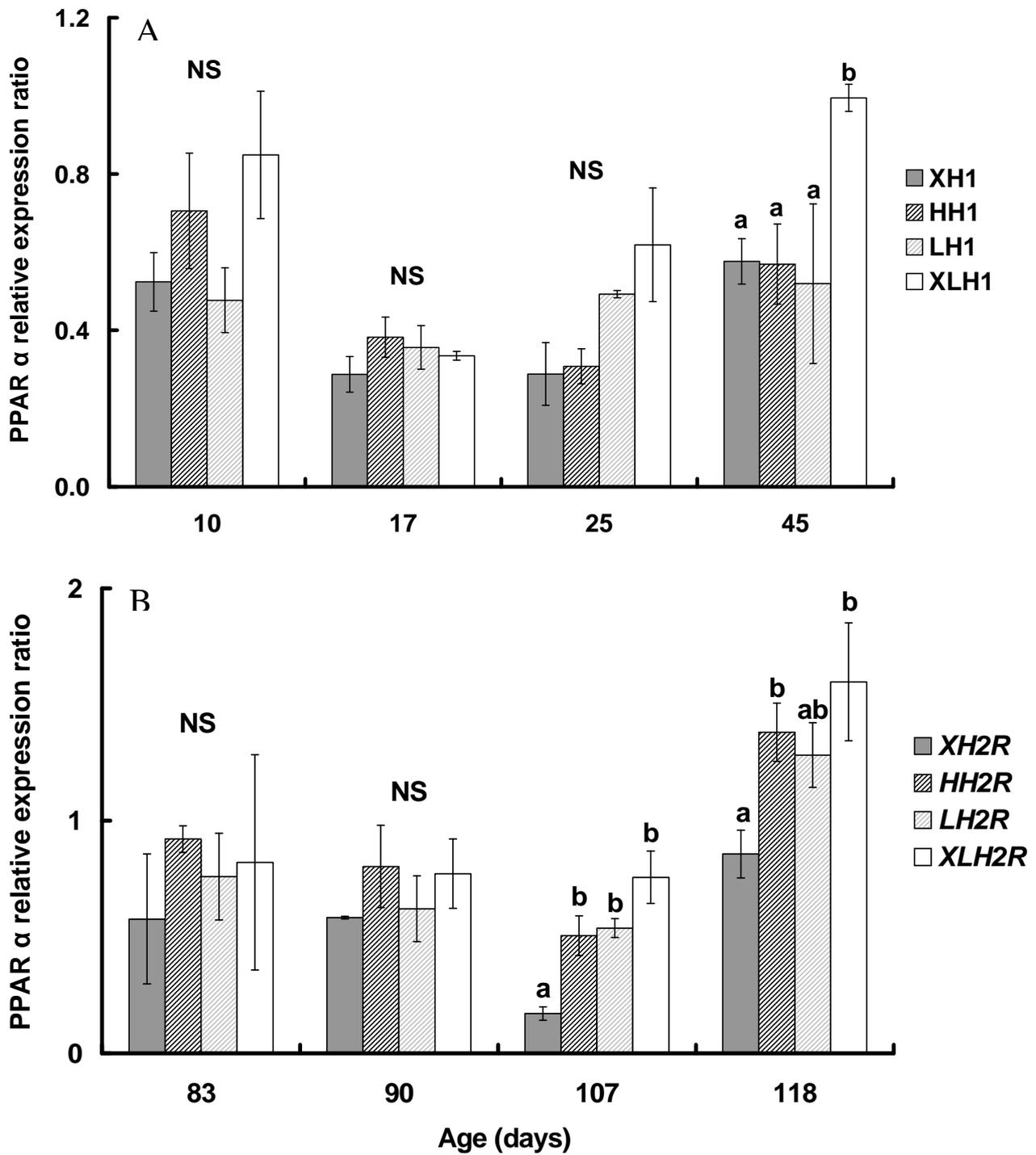


Fig.3. Mean PPAR  $\alpha$  expression ratio ( $\pm$  SE) relative to HH1 (A) and HH2<sub>R</sub> groups (B) according to time in larval (A) and juvenile (B) experiment and for each experimental condition (n=4). For each date, NS indicates non significant differences between groups and <sup>a</sup>, <sup>b,c</sup> differing letters denote significant difference at P<0.05.

Fig.4 ; Vagner et al.

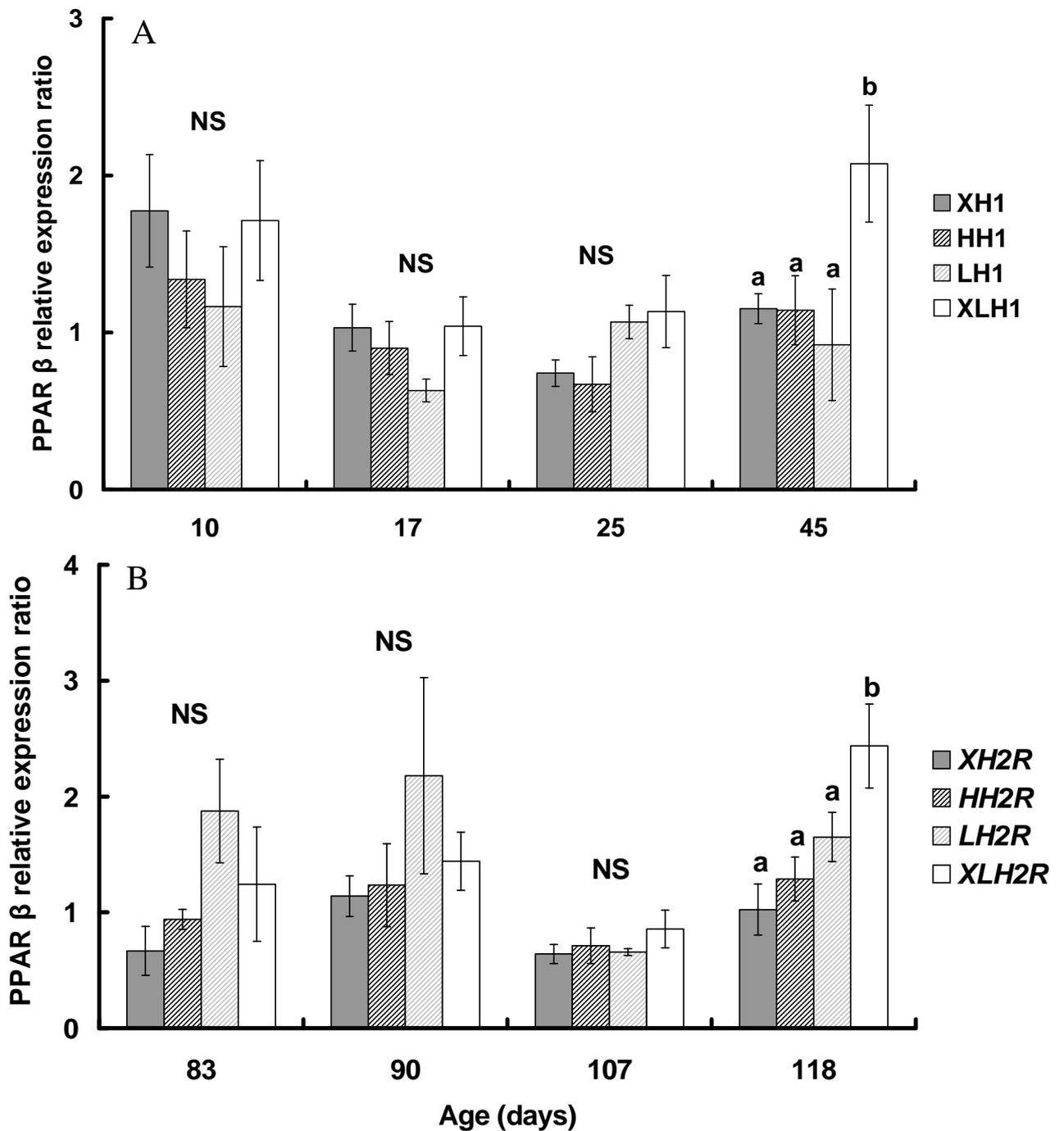


Fig.4. Mean PPAR  $\beta$  expression ratio ( $\pm$  SE) relative to HH1 (A) and HH2<sub>R</sub> (B) according to time in larval (A) and juvenile (B) experiment and for each experimental condition (n=4). For each date, NS indicates non significant differences between groups and <sup>a, b, c</sup> differing letters denote significant difference at P<0.05.

Fig.5 ; Vagner et al.

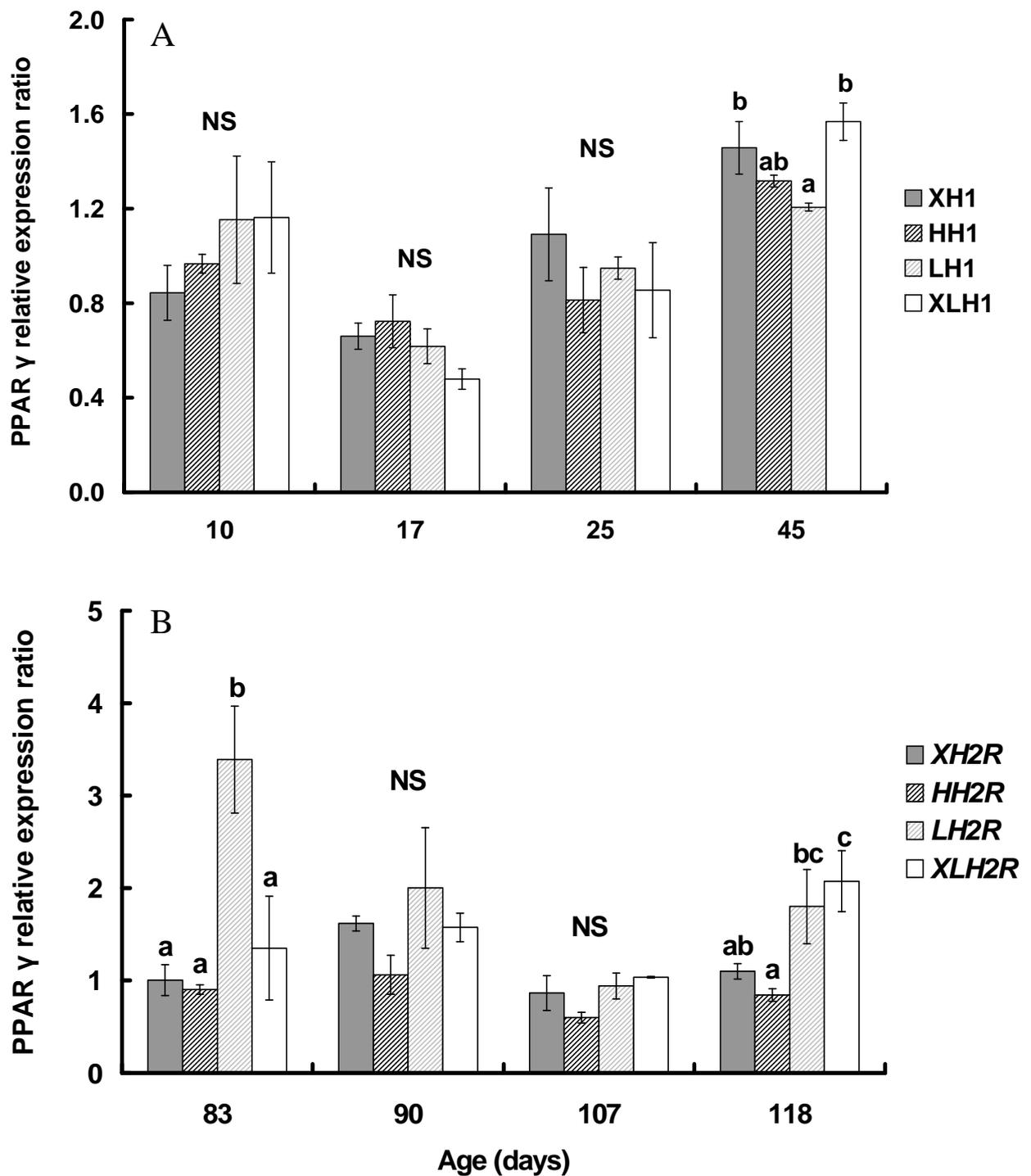


Fig.5. Mean PPAR  $\gamma$  expression ratio ( $\pm$  SE) relative to HH1 (A) and HH2<sub>R</sub> (B) according to time in larval (A) and juvenile (B) experiment and for each experimental condition (n=4). For each date, NS indicates non significant differences between groups and <sup>a, b, c</sup> differing letters denote significant difference at P<0.05.

Fig.6 ; Vagner et al.

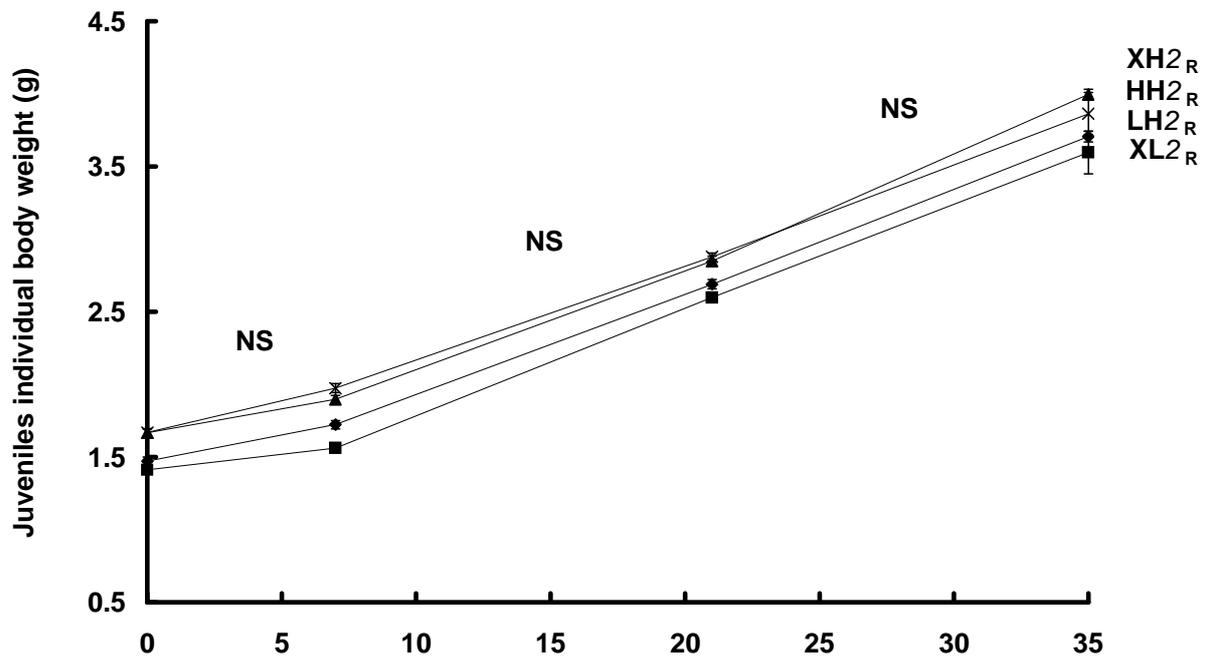


Fig.6. Mean fresh body weight ( $\pm$ SE) increase over time (d-83-118) for the 4 R-groups (n=4). NS indicates non significant differences between groups.

Fig.7; Vagner et al.

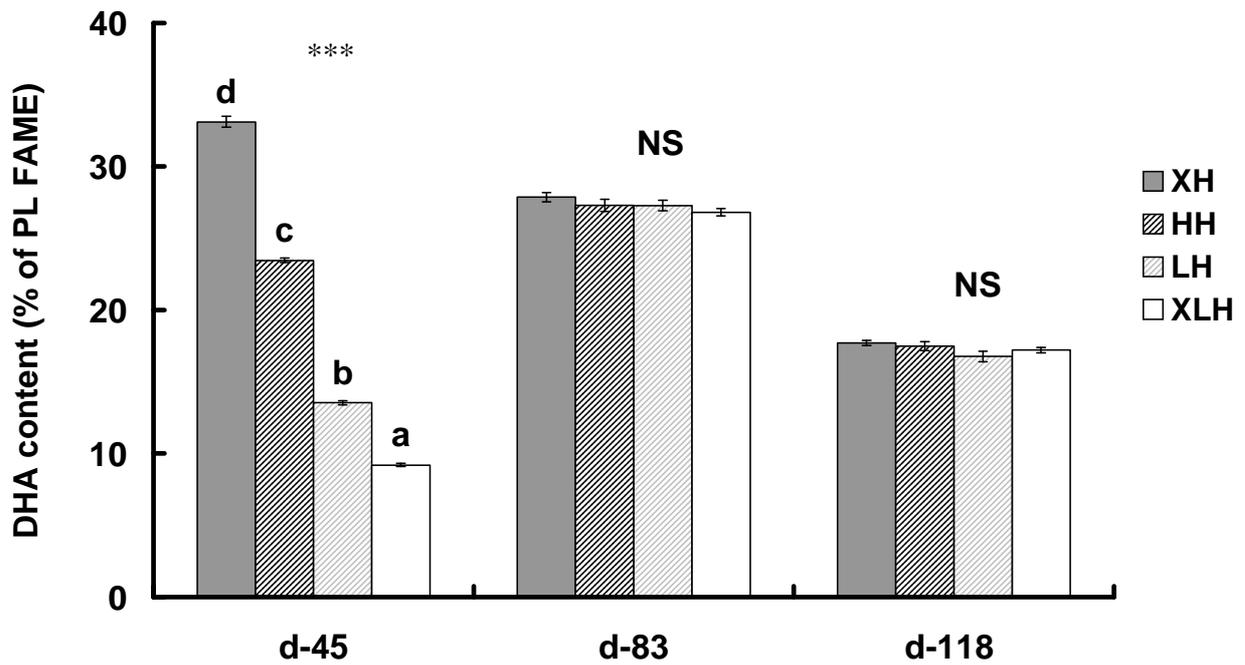


Fig.7. DHA content in PL of d-45 larvae (n=4), d-83 (n=4) and d-118 juveniles fed the R-diet (n=4), according to the larval initial diet. Statistical effect of initial diet is indicated (NS non significant, \*\*\*P<0.001) for each date. <sup>a, b, c, d</sup> differing letters denote significant difference at P<0.05 for each date.

Table 1 ; Vagner *et al.*

Table 1. Formulation (g.100 g<sup>-1</sup>), chemical composition (% DM) and fatty acid composition in TL (% FAME) of the four experimental larval diets (XH, HH, LH and XLH) used in the larval experiment (exp.1).

<i>Ingredients</i> <sup>†</sup>	XH	HH	LH	XLH
Fish meal LT 94	11	11	11	11
Defatted fish meal	41	41	41	41
CPSP 90	11	11	11	11
Soy oil	0	0	1	1
Soy lecithin	7	16	21	23
Marine lecithin LC 40	19	9	2	0
Vitamin mixture <sup>††</sup>	7	7	7	7
Mineral mixture <sup>§</sup>	3	3	3	3
Betaine	1	1	2	2
Cellulose	1	1	1	1
<i>Chemical composition</i>				
Dry matter (%)	91.4	91.0	90.3	90.7
Crude protein (% DM)	63.9	59.2	57.0	57.1
Crude fat (% DM)	18.5	19.1	19.9	20.2
Ash (% DM)	13.9	13.9	14.0	13.9
HUFA n-3 (% DM)	3.8	1.8	0.8	0.5
EPA+DHA (% DM)	3.7	1.7	0.7	0.5
<i>Fatty acids composition in TL</i>				
18:2n-6	18.6	35.2	44.6	47.2
18:3n-6	0.1	0.3	0.1	0.2
20:4n-6	1.7	0.8	0.5	0.2
18:3n-3	1.9	3.3	4.1	4.4
20:5n-3	9.1	4.7	2.2	1.7
22:6n-3	20.5	9.8	3.9	2.2
Σ saturated	27.5	26.1	24.9	24.1
Σ mono-unsaturated	18.5	18.1	18.7	18.7
Σ n-6	20.8	36.7	45.4	48.0
Σ n-3	32.9	19.0	11.0	9.2

<sup>†</sup> Sources: fish meal LT 94: Norse (Fyllingsdalen, Norway); hydrolysed fish meal: Archimex (Vannes, France); fish protein hydrolysate CPSP 90: Sopropêche (Boulogne sur mer, France); soy oil: Système U (Créteil, France); soy lecithin: Louis François (Saint-Maur, France); marine lecithin LC 60: Phosphotech (Saint-Herblain, France).

<sup>††</sup> Vitamin mixture (g kg<sup>-1</sup> vitamin mix): retinyl acetate, 1; cholecalciferol, 2.5; DL- $\alpha$ -tocopheryl acetate, 5; menadione, 1; thiamine-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.

<sup>§</sup> Mineral mixture (g kg<sup>-1</sup> mineral mix): KCL, 90; KI, 0.04; CaHPO<sub>4</sub> 2H<sub>2</sub>O, 500; NaCl, 40; CuSO<sub>4</sub> 5H<sub>2</sub>O, 3; ZnSO<sub>4</sub> 7H<sub>2</sub>O, 4; CoSO<sub>4</sub>, 0.02; FeSO<sub>4</sub> 7H<sub>2</sub>O, 20; MnSO<sub>4</sub> H<sub>2</sub>O, 3; CaCO<sub>3</sub>, 215; MgOH, 124; Na<sub>2</sub>SeO<sub>3</sub>, 0.03; NaF, 1.

Table 2; Vagner et al.

Table 2. Formulation (g.100 g<sup>-1</sup>), chemical composition (%DM) and fatty acid composition in total lipid (% fatty acid methyl esters FAME) of the experimental HUFA-restricted diet (R-diet) and the HUFA-control diet (C-diet) used in the juvenile experiment (exp. 2).

<i>Ingredients</i> <sup>†</sup>	R-diet	C-diet
Lupin without pellicle	50	50
Fish meal LT 94	12	12
Defatted fish meal	8	8
Wheat amygluten 110	7	7
Fish hydrolysate CPSP 90	8	8
Vitamin mixture <sup>††</sup>	1	1
Mineral mixture <sup>§</sup>	1	1
Betaine	0.5	0.5
Methionine	0.2	0.2
Precooked starch	3.7	3.7
Soy lecithin	2	2
Rapeseed oil	6.6	0
Cod-liver oil	0	6.6
<i>Chemical composition</i>		
Dry matter (%)	92.2	92.0
Crude protein (% DM)	51.8	52.2
Crude fat (% DM)	14.9	15.6
Ash (% DM)	6.5	6.5
n-3 HUFA (% DM)	0.4	1.6
EPA+DHA (% DM)	0.3	1.4
<i>Fatty acids composition in TL</i>		
18:2n-6	20.2	11.3
18:3n-6	0.1	0.1
20:4n-6	0.1	0.3
18:3n-3	8.5	4.3
20:5n-3	1.2	4.8
22:6n-3	1.6	6.1
Σ saturated	13.2	17.8
Σ mono-unsaturated	54.3	52.5
Σ n-6	20.7	12.3
Σ n-3	11.9	17.5

<sup>†</sup> Sources: lupin without pellicle: Le Guessant® aquaculture (Lamballe, France); fish meal LT 94: Norse (Fyllingsdalen, Norway); wheat amygluten 110: Chamtor Vitalor (Bazancourt, France); fish protein hydrolysate CPSP 90: Soppêche (Boulogne sur mer, France); precooked starch: Prégéflo Roquette frères (Lestrem, France).

<sup>††</sup> Vitamin mixture (g kg<sup>-1</sup> vitamin mix): retinyl acetate, 1; cholecalciferol, 2.5; DL- $\alpha$ -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.

<sup>§</sup> Mineral mixture (g kg<sup>-1</sup> mineral mix): KCL, 90; KI, 0.04; CaHPO<sub>4</sub> 2H<sub>2</sub>O, 500; NaCl, 40; CuSO<sub>4</sub> 5H<sub>2</sub>O, 3; ZnSO<sub>4</sub> 7H<sub>2</sub>O, 4; CoSO<sub>4</sub>, 0.02; FeSO<sub>4</sub> 7H<sub>2</sub>O, 20; MnSO<sub>4</sub> H<sub>2</sub>O, 3; CaCO<sub>3</sub>, 215; MgOH, 124; Na<sub>2</sub>SeO<sub>3</sub>, 0.03; NaF, 1.

Table 3 ; Vagner *et al.*

Table 3. Total lipid content (TL in % wet weight WW), polar lipid (PL in % TL) and FA profiles (in % FAME) of neutral lipids (NL) and PL in d-45 larvae. Values are mean  $\pm$  SE (n=4). Statistical significance of diet is indicated (NS no significant; \* P<0.05, \*\* P<0.01 and \*\*\* P<0.001). Values not sharing a common letter in the same line are significantly different.

	D-45 larval composition								Statistical analysis
	XH/		HH/		LH/		XLH/		
	mean	SE	mean	SE	mean	SE	mean	SE	
TL (% WW)	2.3	0.2	2.9	0.2	2.9	0.2	2.7	0.2	NS
PL (% TL)	47 <sup>a</sup>	4	44 <sup>ab</sup>	2	42 <sup>b</sup>	0	41 <sup>b</sup>	1	*
NL									
16:0	18.9 <sup>a</sup>	0.3	19.2 <sup>a</sup>	0.2	17.9 <sup>b</sup>	0.2	17.4 <sup>b</sup>	0.4	***
18:0	4.4 <sup>a</sup>	0.1	3.9 <sup>b</sup>	0.2	4.4 <sup>a</sup>	0.2	4.6 <sup>a</sup>	0.2	***
18:1	0.2 <sup>a</sup>	0.1	0.2 <sup>ab</sup>	0.2	0.1 <sup>b</sup>	0.1	0.0 <sup>b</sup>	0.1	*
18:2n-6	18.7 <sup>a</sup>	0.1	36.0 <sup>b</sup>	0.1	43.4 <sup>c</sup>	0.2	44.4 <sup>d</sup>	0.3	***
18:3n-6	0.2 <sup>a</sup>	0.0	0.1 <sup>b</sup>	0.0	0.5 <sup>c</sup>	0.0	0.9 <sup>d</sup>	0.1	**
20:2n-6	1.2 <sup>a</sup>	0.0	1.4 <sup>b</sup>	0.1	1.3 <sup>ab</sup>	0.0	1.2 <sup>a</sup>	0.1	*
20:4n-6	1.4 <sup>a</sup>	0.0	0.7 <sup>b</sup>	0.0	0.3 <sup>c</sup>	0.0	0.2 <sup>d</sup>	0.0	**
18:3n-3	1.8 <sup>a</sup>	0.0	3.1 <sup>b</sup>	0.0	3.7 <sup>c</sup>	0.0	3.8 <sup>c</sup>	0.1	***
18:4n-3	0.5 <sup>a</sup>	0.0	0.4 <sup>ab</sup>	0.0	0.4 <sup>ab</sup>	0.0	0.4 <sup>b</sup>	0.0	*
20:4n-3	0.4 <sup>a</sup>	0.0	0.2 <sup>b</sup>	0.0	0.2 <sup>b</sup>	0.0	0.2 <sup>b</sup>	0.0	***
20:5n-3	7.7 <sup>a</sup>	0.1	3.9 <sup>b</sup>	0.1	1.8 <sup>c</sup>	0.1	1.1 <sup>d</sup>	0.0	***
22:5n-3	0.7 <sup>a</sup>	0.0	0.5 <sup>b</sup>	0.0	0.3 <sup>c</sup>	0.0	0.3 <sup>c</sup>	0.0	***
22:6n-3	18.2 <sup>a</sup>	0.2	7.3 <sup>b</sup>	0.2	2.3 <sup>c</sup>	0.0	1.4 <sup>d</sup>	0.0	***
Σ saturated	26.8 <sup>a</sup>	0.2	25.4 <sup>b</sup>	0.2	24.6 <sup>c</sup>	0.1	24.4 <sup>c</sup>	0.1	***
Σ MUFAs	22.2 <sup>a</sup>	0.1	20.7 <sup>c</sup>	0.3	21.1 <sup>bc</sup>	0.0	21.4 <sup>b</sup>	0.0	***
Σ PUFAs	51.0 <sup>a</sup>	0.4	53.9 <sup>b</sup>	0.3	54.3 <sup>b</sup>	0.3	54.1 <sup>b</sup>	0.3	***
PL									
16:0	22.6 <sup>a</sup>	0.2	21.6 <sup>b</sup>	0.2	20.1 <sup>c</sup>	0.4	19.8 <sup>c</sup>	0.3	***
18:0	6.2 <sup>a</sup>	0.3	6.6 <sup>b</sup>	0.1	7.2 <sup>c</sup>	0.2	7.5 <sup>c</sup>	0.1	***
18:1	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.1	NS
18:2n-6	8.8 <sup>a</sup>	0.1	22.6 <sup>b</sup>	0.1	34.9 <sup>c</sup>	0.2	39.6 <sup>d</sup>	0.2	***
18:3n-6	0.0 <sup>a</sup>	0.0	0.2 <sup>b</sup>	0.1	0.3 <sup>c</sup>	0.1	0.5 <sup>c</sup>	0.0	***
20:2n-6	1.3 <sup>a</sup>	0.1	1.7 <sup>b</sup>	0.1	1.8 <sup>b</sup>	0.0	1.9 <sup>b</sup>	0.0	***
20:3n-6	0.1 <sup>a</sup>	0.0	0.1 <sup>a</sup>	0.0	0.2 <sup>b</sup>	0.0	0.2 <sup>b</sup>	0.0	*
20:4n-6	3.1 <sup>a</sup>	0.0	1.9 <sup>b</sup>	0.0	1.0 <sup>c</sup>	0.0	0.6 <sup>d</sup>	0.1	***
18:3n-3	0.5 <sup>a</sup>	0.0	1.2 <sup>b</sup>	0.0	1.7 <sup>c</sup>	0.0	1.9 <sup>d</sup>	0.0	***
20:5n-3	9.7 <sup>a</sup>	0.1	6.8 <sup>b</sup>	0.0	4.7 <sup>c</sup>	0.1	3.7 <sup>d</sup>	0.1	***
22:5n-3	0.5 <sup>a</sup>	0.0	0.6 <sup>ab</sup>	0.0	0.6 <sup>b</sup>	0.0	0.6 <sup>b</sup>	0.0	*
22:6n-3	33.1 <sup>a</sup>	0.4	23.5 <sup>b</sup>	0.2	13.5 <sup>c</sup>	0.2	9.2 <sup>d</sup>	0.1	***
Σ saturated	29.8 <sup>a</sup>	0.3	29.1 <sup>a</sup>	0.1	28.2 <sup>b</sup>	0.3	28.1 <sup>b</sup>	0.2	**
Σ MUFAs	12.7 <sup>ab</sup>	0.3	12.1 <sup>a</sup>	0.3	12.6 <sup>ab</sup>	0.1	13.0 <sup>b</sup>	0.0	*
Σ PUFAs	57.6 <sup>a</sup>	0.7	58.8 <sup>b</sup>	0.3	59.2 <sup>b</sup>	0.4	58.9 <sup>b</sup>	0.3	*

Table 4 ; Vagner *et al.*

Table 4. Total lipid content (TL in % wet weight WW), polar lipid (PL in % TL) and FA profiles (in % FAME) of neutral lipids (NL) and PL in each treatment of d-118 R-groups. Values are mean  $\pm$  SE (n=4). Statistical significance of diet is indicated (NS no significant; \* P<0.05). Values not sharing a common letter in the same line are significantly different.

	D-118 juveniles								Statistical analysis
	XH2 <sub>R</sub>		HH2 <sub>R</sub>		LH2 <sub>R</sub>		XLH2 <sub>R</sub>		
	mean	SE	mean	SE	mean	SE	mean	SE	
TL (% WW)	9.8	0.6	8.8	0.2	8.8	0.5	9.3	0.2	NS
PL (% TL)	13.6	1.0	14.2	0.8	11.6	0.9	14.1	1.0	NS
NL									
16:0	11.4	0.1	11.8	0.1	11.4	0.1	11.9	0.1	NS
18:0	2.9	0.0	3.0	0.0	2.9	0.0	3.0	0.0	NS
18:1	0.1	0.0	0.1	0.0	0.1	0.1	0.1	0.0	NS
18:2n-6	16.2	0.2	16.6	0.3	17.0	0.1	16.8	0.1	NS
18:3n-6	0.3	0.0	0.3	0.0	0.3	0.0	0.3	0.0	NS
20:2n-6	0.6	0.0	0.6	0.0	0.6	0.0	0.6	0.0	NS
20:3n-6	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	NS
20:4n-6	0.2	0.0	0.2	0.0	0.2	0.0	0.2	0.0	NS
18:3n-3	6.2	0.1	6.3	0.2	6.5	0.0	6.4	0.0	NS
18:4n-3	0.6	0.0	0.6	0.0	0.6	0.0	0.6	0.0	NS
20:3n-3	0.1 <sup>a</sup>	0.0	0.1 <sup>b</sup>	0.0	0.1 <sup>b</sup>	0.0	0.1 <sup>b</sup>	0.0	**
20:4n-3	0.2	0.0	0.2	0.0	0.2	0.0	0.2	0.0	NS
20:5n-3	2.4 <sup>a</sup>	0.0	2.1 <sup>b</sup>	0.1	2.1 <sup>b</sup>	0.1	2.0 <sup>b</sup>	0.1	*
22:5n-3	0.5 <sup>a</sup>	0.0	0.4 <sup>ab</sup>	0.0	0.4 <sup>b</sup>	0.0	0.4 <sup>b</sup>	0.0	*
22:6n-3	2.8 <sup>a</sup>	0.1	2.3 <sup>b</sup>	0.1	2.2 <sup>b</sup>	0.1	2.3 <sup>b</sup>	0.1	*
Σ saturated	17.7	0.2	17.6	0.4	17.1	0.1	17.2	0.2	NS
Σ MUFAs	51.9	0.2	52.7	0.3	52.7	0.2	52.8	0.2	NS
Σ PUFAs	30.3	0.2	29.7	0.3	30.2	0.2	30.0	0.2	NS
PL									
16:0	15.4	0.1	15.5	0.2	15.3	0.1	15.6	0.1	NS
18:0	7.2	0.1	7.4	0.1	7.2	0.1	7.2	0.0	NS
18:1	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	NS
18:2n-6	13.1	0.2	13.3	0.2	13.4	0.3	13.5	0.2	NS
18:3n-6	0.4	0.0	0.4	0.0	0.4	0.0	0.4	0.0	NS
20:2n-6	0.9	0.0	1.0	0.0	1.0	0.0	1.0	0.1	NS
20:3n-6	0.3	0.0	0.3	0.0	0.3	0.0	0.2	0.0	NS
20:4n-6	1.5	0.0	1.5	0.0	1.5	0.0	1.5	0.0	NS
18:3n-3	3.4	0.1	3.5	0.1	3.4	0.1	3.5	0.1	NS
18:4n-3	0.3	0.0	0.3	0.0	0.3	0.0	0.3	0.0	NS
20:3n-3	0.1	0.0	0.1	0.0	0.2	0.0	0.1	0.0	NS
20:4n-3	0.2 <sup>a</sup>	0.0	0.2 <sup>a</sup>	0.0	0.3 <sup>b</sup>	0.0	0.2 <sup>a</sup>	0.0	*
20:5n-3	6.6	0.1	6.5	0.2	6.4	0.1	6.5	0.1	NS
22:5n-3	1.1	0.0	1.1	0.0	1.1	0.0	1.1	0.0	NS
22:6n-3	17.7	0.2	17.5	0.4	16.8	0.6	17.2	0.2	NS
Σ saturated	24.6	0.2	24.8	0.3	24.7	0.2	24.7	0.3	NS
Σ MUFAs	29.8	0.1	29.7	0.3	30.3	0.3	29.7	0.1	NS
Σ PUFAs	45.6	0.1	45.5	0.4	45.0	0.4	45.6	0.2	NS