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Effects of Dietary Arachidonic and Eicosapentaenoic Acids on Common Dentex  
(Dentex dentex Linnaeus 1758) Larval Performance

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19 **Abstract**

20 **Oily emulsions containing constant levels of total fatty acids (FA) and varying**  
21 **eicosapentaenoic acid (EPA) and arachidonic acid (ARA) were used to enrich**  
22 **rotifers. Common dentex larval survival and growth were compared among groups**  
23 **fed the different enriched live prey. Growth, survival rate and lipid composition of**  
24 **larvae suggest that feeding common dentex the first 15 days post-hatching with 2.5**  
25 **– 3% EPA, 6-8% DHA and DHA/EPA ratio of 2.0 – 2.5 is sufficient to fulfil their**  
26 **EPA requirements. Higher amounts of dietary EPA did not result in any significant**  
27 **improvement in growth or survival. EPA requirement during this period of larval**  
28 **development does not seem to be as critical as other fatty acids during the first 15**  
29 **days of common dentex larval development, but it does not exclude its essentiality**  
30 **later in development. In the case of ARA, nutritional requirements are low**  
31 **compared to other marine finfish species, with the upper limit of this essential fatty**  
32 **acid around 2% of total fatty acids provided in the live prey composition.**

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35 **Keywords: Common dentex larvae, Dentex dentex larvae, eicosapentaenoic acid,**  
36 **arachidonic acid, essential fatty acids, lipid nutrition**

## Introduction

38

39 Common dentex (Dentex dentex Linnaeus, 1758) has been considered a candidate  
40 species for the Mediterranean finfish aquaculture diversification. One of the main  
41 bottlenecks for scaling up its culture at industrial production is the high mortality during  
42 its larval rearing (Sweetman 1992; Abellán and Basurco 1999). Inadequate culturing  
43 conditions, unsuitable feeding nutritional demands not covered are considered the most  
44 probable causes of such mortality (Rigos et al. 1998; Abellán 2000; Crespo et al. 2001;  
45 Rueda & Martínez 2001).

46 Saturated and monounsaturated fatty acids (FA) can be biosynthesized de novo by  
47 all living organisms while polyunsaturated fatty acids (PUFA) can only be biosynthesized  
48 de novo by photosynthetic organisms (Sargent, 1976). PUFA requirements of non-  
49 photosynthetic organisms, including fish larvae, must be fulfilled by their diet; due to  
50 PUFA role in certain metabolic pathways or functions they are called “essential fatty  
51 acids”, EFA. Arachidonic acid (ARA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3)  
52 and docosahexaenoic acid (DHA, 22:6n-3) are essential fatty acids which, in strict  
53 carnivores as cats, are mainly provided by the diet because the activity of their *de novo*  
54 biosynthesis enzymes is very low (Tocher, 2003; Bell et al., 2003). Common dentex  
55 (Dentex dentex Linnaeus 1758) larvae are also strict carnivores (Rueda & Martínez,  
56 2001), consequently, their EFA dietary requirements can be considered a priori very high.

57 Dietary requirements of EFA are species-specific, age-specific and depend not  
58 only on the total amount (% of total FA or total lipids) of each FA, but also on their ratios.  
59 Arachidonic acid, EPA and DHA interact and compete for enzymes involved in their  
60 metabolic pathways and in their biological functions, making it difficult to study dietary

61 requirements of each EFA isolated from the rest of fatty acids (Sargent, 1976; Sargent et  
62 al., 1999a, 1999b; Izquierdo et al., 2000).

63       Essential fatty acids can be used as a source of energy or structural components  
64 of cell membranes, affecting their physico-chemical properties (Tocher, 2003). They are  
65 essential during all the life span of an organism, but their role as structural components  
66 might be more critical during larval stages since these stages are characterized by the  
67 formation of new tissues and organs (Santamaría, 2001).

68       Arachidonic acid is incorporated into cell membrane phospholipids where it works  
69 as a cell signaling molecule, either in its own right or after its conversion to oxidized  
70 derivatives known as eicosanoids. Proteolytic and hormonal stimuli can activate  
71 arachidonic acid cascades that lead to the production of eicosanoids. Eicosanoids are  
72 highly biologically active metabolites, with well-established roles in many processes in  
73 mammals, including thrombosis, inflammation and immunosuppression (Calder, 2007).  
74 In humans, high dietary ARA levels are also related with the reduction of cardiovascular  
75 diseases (Gershwin et al., 2000) and with some eicosanoid derived types of cancer  
76 (Tocher, 2003).

77       Several authors (Sargent et al., 1999b; Izquierdo et al., 2000; Bell et al., 2003)  
78 have identified EPA as essential for fish larvae, due to its interactions with ARA and  
79 DHA. It has two main roles: as part of phospholipids of the neural and cardiac cell  
80 membranes (Lauritzen et al, 2001), and as a precursor of 3-series eicosanoids; in this last  
81 role, it competes with ARA. Activity of eicosanoids depends upon dietary EPA/ARA  
82 ratio; if there is a high EPA dietary level, then the ARA level is lower and consequently  
83 there are less eicosanoids produced from ARA (Tocher, 2003). This interaction is

84 interesting for balancing the negative effects of ARA derived from eicosanoids and the  
85 positive effect of dietary ARA (Gershwin et al., 2000).

86 Izquierdo & Fernández-Palacios (1997) suggested three ways to investigate the  
87 fatty acid requirement of marine fish larvae: 1) the study of egg and larval composition  
88 at different developmental stages, 2) the comparison of the composition between fed and  
89 starved larvae at the same developmental stage, and 3) the use of feeding experiments  
90 controlling the fatty acid composition of the delivered food. Fatty acid requirements of  
91 common dentex larvae have not yet been determined. However, data on the composition  
92 of eggs and newly hatched larvae (Tulli & Tibaldi, 1997; Tibaldi & Tulli, 1998; Giménez  
93 et al., 2008), as well as the composition of common dentex larvae kept under starvation  
94 (Mourente et al., 1999a; Giménez et al., 2008) or fed (Mourente et al., 1999b; Giménez  
95 et al., 2008) are available. In the present dose-response experiments, the fatty acid  
96 composition of delivered food was controlled and monitored. Oily emulsions containing  
97 constant levels of total FA and varying ARA or EPA were used for rotifer enrichment,  
98 and larval survival, growth and lipid composition were compared among groups fed the  
99 different enriched live prey. The ARA or EPA dietary requirement of the larvae was  
100 identified as the fatty acid level or EFA ratio in the diet that gives the best larval  
101 performance.

## 102 **Materials and Methods**

### 103 Spawning Induction and Egg Quality Assessment

104 Eggs were obtained by photothermal induction of two captive common dentex  
105 broodstock reared at the Institut de Recerca i Tecnologia Agroalimentàries (IRTA)  
106 facilities. A single batch of floating eggs was incubated in one 35-L cylindrical PVC  
107 container with three 10x10 cm lateral windows and bottom of 150- $\mu$ m diameter mesh,

108 provided with an air-lift system and aeration supply (“basket”). This container was  
109 immersed in a 500-L black-bottomed tank connected to a recirculation unit equipped with  
110 mechanical (up to 1  $\mu\text{m}$  diameter), biological and UV filters, and a temperature controller  
111 (Carbó et al., 2002). Larvae hatched after 24 h at  $19 \pm 1$  °C and  $35 \text{ g L}^{-1}$  salinity.  
112 The same batch of eggs was used for the two experiments. Larvae of 3 dph were stocked  
113 at 20 individuals (ind)  $\text{mL}^{-1}$ , light conditions 18L:6D and  $3.4 \mu\text{mol m}^{-2} \text{ s}^{-1}$  irradiance in  
114 the water surface, and fed once daily with 10 ind  $\text{mL}^{-1}$  of enriched Brachionus plicatilis  
115 (Giménez & Estévez 2008a; Fig. 1). The experiments finished when larvae were 15 dph.  
116 Survival, total length (TL), dry weight (DW) and lipid composition of larvae were  
117 measured on larvae of 3 and 15 dph.

#### 118 Experiment for Arachidonic Acid Dietary Requirements

119 Larvae were stocked randomly in eight 100-L white-bottomed tanks connected to  
120 a recirculation unit (Carbó et al., 2002). Tetraselmis chuii ( $55,343 \pm 941$  cells  $\text{mL}^{-1}$ ) was  
121 added to the tanks the day before stocking the larvae. Temperature, salinity, oxygen and  
122 pH were checked daily and their values averaged  $20.73 \pm 1.07$  °C,  $35.47 \pm 0.38 \text{ g L}^{-1}$ ,  $8.65$   
123  $\pm 0.26 \text{ mg L}^{-1}$ ,  $7.99 \pm 0.06$ , respectively. Nitrites and ammonia were checked once per  
124 week and their maximal values were 0.3 and 0.1  $\text{mg L}^{-1}$ , respectively. Brachionus  
125 plicatilis were enriched with the determined emulsion level, two tanks per level: Control,  
126 High, Medium and Low.

#### 127 Experiment for Eicosapentaenoic Acid Dietary Requirements

128 Larvae were stocked randomly into twelve 35-L baskets (as described in the  
129 section on spawning induction and egg quality assessment section) immersed in 500-L  
130 black-bottomed tanks, four containers per tank, connected to the same recirculation unit  
131 (Carbó et al., 2002). Tetraselmis chuii ( $57,351 \pm 730$  cells  $\text{mL}^{-1}$ ) was added to the

132 holding tanks the day before stocking the larvae. Temperature, salinity, oxygen and pH  
133 were checked daily and their values averaged 19.8 +/- 1.2°C, 35.6 +/- 0.3 g L<sup>-1</sup>, 8.7 +/-  
134 0.4 mg L<sup>-1</sup>, 8.0 +/- 0.1, respectively. Nitrites and ammonia were checked once per week  
135 and their maximal values were 0.3 and 0.1 mg L<sup>-1</sup>, respectively. Larvae in the baskets  
136 immersed in the same tank were fed with B. plicatilis enriched with the same EPA  
137 emulsion level: Low, Medium or High.

#### 138 Emulsion Formulation

139 The hypothesis that lipid composition of marine animals might reflect the  
140 composition of the diet (Sargent, 1976) was taken into account for emulsion design.  
141 Following this hypothesis, lipid composition of common dentex larvae (Giménez et al.,  
142 2008) and the published composition of Tisbe sp. (Evjemo & Olsen, 1997) were used as  
143 reference levels for emulsion composition. Tisbe sp. composition was chosen because  
144 this copepod species is a known natural prey for finfish larvae, and was present in the  
145 mesocosmos rearing tanks in previous experiments, in which better larval performance  
146 was obtained (Giménez & Estévez, 2008b).

147 Three levels of essential fatty acids (expressed in % total fatty acids, %TFA) were  
148 defined for the enrichment emulsion: Low, L, below the composition of common dentex  
149 larvae in cultured conditions (2% ARA, 3% EPA); Medium, M, close to the composition  
150 of Tisbe sp. (4% ARA, 15% EPA) and High, H, two times the composition of Tisbe sp.  
151 (8.7% ARA, 30% EPA). Control groups (C) were fed with Easy DHA Selco® (INVE,  
152 Belgium) enriched rotifers. The formulation of the emulsions is shown in Table 1:  
153 Vevodar<sup>TM</sup> oil (DSM Food Specialties, Netherlands) was the main source of ARA,  
154 Neuromins<sup>TM</sup> oil (Martek Bioscience Corporation, USA) provided mainly DHA, and  
155 EPA500<sup>TM</sup> oil (Croda International Plc., UK) was the main source of EPA. Olive oil



156 variety Cornicabra, rich in palmitic (16:0) and oleic acids (18:1), whereas corn oil  
157 provided mainly linoleic acid (18:2n-6). Soy lecithin was added as emulsifier, and  $\alpha$ -  
158 tocophenol (Sigma Aldrich Co., Germany) was required as antioxidant. The emulsions  
159 were stored at 4°C.

#### 160 Rotifer Enrichment

161 Rotifers (Brachionus plicatilis SM morphotype) were cultured at 20°C, 33 g L<sup>-1</sup>  
162 salinity and 24hL:0hD, in 100-L cylindroconical metacrylate tanks using T. chuii and  
163 baker's yeast as food. Every day, population density in the culturing tank was checked,  
164 and the amount needed for larval feeding was harvested using a 60- $\mu$ m diameter mesh  
165 immersed in a bucket filled with UV-filtered seawater. They were gently rinsed with UV-  
166 filtered seawater and restocked to the volume of UV-filtered seawater needed for  
167 enrichment purposes.

168 The enrichment protocol was: 250 ind mL<sup>-1</sup>, 0.1 g emulsion L<sup>-1</sup>, during 2 h at 20°C  
169 with continuous light and aeration. After enrichment, rotifers were filtered using a 60- $\mu$ m  
170 diameter mesh immersed in a bucket filled with UV-filtered seawater. There, rotifers were  
171 gently rinsed with UV- filtered seawater, followed by a 2 min UV-filtered tap water  
172 washing, and finally restocked to UV-filtered seawater before addition to experimental  
173 tanks.

#### 174 Samples and Data Collection

175 Growth data were obtained from a pool of 20 larvae per age and experimental  
176 replicate. Larvae were first lightly anesthetised using aminobenzoic acid (MS 222,  
177 SIGMA) and the distance between mouth (upper jaw) and the end of the notochorda  
178 (Standard Length, SL) was measured with a Nikon SMZ800 dissecting microscope  
179 (Nikon, Spain) connected to an Olympus DP25 digital camera (Olympus Corporation,

180 Germany) and image analysis software (AnalySIS GmbH, Olympus, Germany) to the 0.01  
181 mm. The same individuals were then sacrificed by MS 222 excess, filtered using a 150-  
182  $\mu\text{m}$  hand-made mesh, gently rinsed with tap water and distilled water. Excess water was  
183 released and dried. Larvae were weighed (Wet Weight, WW), oven-dried at 60° C for 24  
184 h, and weighed again to obtain Dry Weight (DW) to the nearest  $\mu\text{g}$  on a Mettler A-20  
185 microbalance (Mettler Toledo, Columbus, OH, USA). Averaged water percentage (%W)  
186 was estimated from these data.

187 Larvae sampled for lipid analysis were sacrificed by MS 222 excess, filtered using  
188 a 150- $\mu\text{m}$  hand-made mesh, gently rinsed with tap water and distilled water. Excess water  
189 was released and dried. Sampled larvae were counted when possible, total amount  
190 weighed and stored in 2-mL glass vials at -80° C until analysis. Water percentage obtained  
191 from weight growth data was used to estimate water amount in lipid samples.

192 Rotifers were filtered using a 50- $\mu\text{m}$  hand-made mesh, gently rinsed with tap  
193 water and distilled water. Excess water was released and dried. Samples for lipid analysis  
194 were weighed and stored in 2-mL glass vials at -80° C until analysis. For each sample for  
195 lipid analysis, four subsamples were used to estimate WW, DW and %W as described  
196 above for fish larvae. Rotifers were sampled before and just after the enrichment protocol.  
197 An amount of enrichment emulsions was weighed and stored in 2-mL glass vials at -80°  
198 C until lipid analysis; WW, DW and W% were estimated as well.

#### 199 Lipid analysis

200 Total lipids were extracted from samples by homogenization in  
201 chloroform/methanol (2:1, v/v) (Folch et al., 1957) and quantified gravimetrically after  
202 evaporation of the solvent under a stream of nitrogen followed by vacuum desiccation  
203 overnight.

204 Fatty acids were methylated following the acid catalyzed transmethylation method  
205 used by Christie (1982). They were extracted twice using isohexane: diethyl ether (1:1,  
206 v/v), purified on TLC plates and analyzed using a Fisons GC 8000 gas chromatograph  
207 (Carlo Erba, Milan, Italy) equipped with a capillary column (ZB Wax, 60 m x 0.32 mm  
208 i.d.; Phenomenex, Macclesfield, UK) and a flame ionization detector (Tocher and Harvie,  
209 1988). Sample application was by on-column injection, and hydrogen was used as the  
210 carrier gas. During the course of each analysis, the oven was programmed to increase  
211 from 50 to 150° C at a rate of 40° C min<sup>-1</sup> and then to a final temperature of 225° C at a  
212 rate of 1.5° C min<sup>-1</sup>. Peaks were identified by comparison with well-characterized  
213 standards (Ackman, 1980) and a well-characterized fish oil, and quantified by means of  
214 the response factor to the internal standard, 17:0 fatty acid, added before methylation.

#### 215 Statistical Analysis

216 Results were analyzed separately for each experimental setup, by one-way  
217 analysis of variance (ANOVA, P<0.05) and a post hoc pairwise multiple comparison of  
218 the means using Tukey's test (P<0.05, STATGRAPHICS PLUS 4.1, Microsoft Inc.).

### 219 **Results and Discussion**

#### 220 Fatty Acid Composition of the Delivered Rotifer

221 Fatty acid composition of enriched live food depends not only on the mixture of  
222 oils, but the emulsifier use or the enrichment time (Estévez & Giménez 2017). Rotifers  
223 are able to metabolize the FA to some extent, and are known to lose their FA composition  
224 a few hours after enrichment (Romero-Romero & Yúfera 2012). The composition of the  
225 emulsions (Table 2) and the rotifers after enrichment (Table 3) were analyzed and  
226 compared in order to check if rotifer composition matched the emulsion composition  
227 when delivered to the larvae. Levels of ARA and EPA in rotifers changed according to

228 the enrichment emulsion used. Rotifers enriched with varying levels of ARA showed the  
229 same gradation of Low-, Medium- and High-levels, at similar % as in the emulsions; the  
230 emulsion – rotifer percentages were 1.6% – 1.6% for Low, 3.3% – 2.8% for Medium and  
231 7.6% - 6.2% for High. The percentage of EPA in rotifers enriched with varying levels of  
232 ARA was low (0.4% to 0.7%), similar between rotifers enriched with ARA emulsions  
233 and lower than in rotifers enriched with EPA emulsions. Rotifers enriched with Easy  
234 DHA Selco® presented the lowest level of ARA (0.6%, Table 3) and the highest level of  
235 EPA (4.8%, Table 3) of all the rotifers delivered in the experiment for ARA dietary  
236 requirements; this level was between those obtained with the emulsions EPA-Low and  
237 EPA-Medium in the rotifers delivered in the experiment for EPA dietary requirements.

238 Rotifers enriched with varying levels of EPA showed the same gradation of Low-  
239 , Medium- and High-levels, but slightly different % as in the emulsions; the emulsion –  
240 rotifer percentages were 2.6% – 2.6% for Low, 13.3% – 8.8% for Medium and 29.4% -  
241 16.7% for High. The percentage of ARA in rotifers enriched with varying levels of EPA  
242 were similar between EPA emulsions (3.4% to 3.5%) and to ARA-Medium emulsion.

243 When the percentage of a specific EFA is modified in the diet, other important  
244 fatty acids vary. Substrates for  $\beta$ -oxidation (SFA and MUFA) and DHA should be also  
245 taken into account. In this experiment, there were no differences in the percentage of SFA  
246 between ARA diets (only when they were compared to the non enriched rotifer) neither  
247 between EPA diets (Table 3). The percentage of MUFA were not different between ARA  
248 diets, but did differ in EPA diets (Table 3). Regarding the percentage of DHA, there were  
249 no differences between the diets used in the same setup (Table 3), therefore, the  
250 differences observed among the experimental groups were not due to the level of this  
251 EFA in the diet.

252 Rotifer composition after the enrichment and optimal rotifer:larvae ratio for the  
253 experimental design (Giménez & Estévez 2008a) can be controlled, but there is no control  
254 about the actual amount of rotifers ingested per larvae or by any individual larva, or time  
255 of ingestion (how long after the enrichment, i.e. level of enrichment loss). This variability  
256 is inherent to work with finfish larvae and live prey, but it must be taken into account that  
257 results about larval performance are not individual values but pools of several larvae from  
258 each experimental group.

#### 259 Experiment for Arachidonic Acid Dietary Requirements

260 The significantly higher survival rate (Table 4) found in the larvae fed low dietary  
261 ARA levels (rotifers “Easy DHA Selco®” and “ARA-L”, Table 3) suggests a negative  
262 effect of this EFA during early larval stages of common dentex development. The best  
263 performance was obtained in the control group, fed with rotifers enriched with Easy DHA  
264 Selco®, with 5.7% survival rate, and significantly higher growth ( $215.6 \pm 50.2 \mu\text{g}$  and  
265  $4.8 \pm 0.8\text{mm}$ , average  $\pm$  SD). Larvae fed rotifers enriched with ARA-L emulsions showed  
266 a similar survival rate (5.6%) but lower growth ( $150.4 \pm 29.3 \mu\text{g}$  and  $4.3 \pm 0.5 \text{mm}$ ). Larvae  
267 fed with rotifers enriched with ARA-M and ARA-H emulsions presented a decreasing  
268 survival rate, and low growth, similar to ARA-L. The differences observed in larval  
269 growth between control and ARA-L fed groups, can be due to: 1) different levels of ARA,  
270 EPA and EPA/ARA ratio in the enriched rotifers (Table 3), or 2) other essential  
271 compounds in Easy DHA Selco® composition (i.e. hydrosoluble vitamins and other  
272 additives) that are not present in the experimental emulsions. Rotifers enriched with Easy  
273 DHA Selco® showed the lowest ARA levels (0.6%) and the highest EPA levels (4.8%)  
274 of the emulsions used in this experiment, consequently, the EPA/ARA ratio was the  
275 highest. Compared to these rotifers, those enriched with ARA-L emulsion showed higher

276 ARA levels (1.6%), lower EPA levels (0.6%, similar to all rotifers enriched with ARA  
277 emulsions) and lower EPA/ARA ratios (0.4). Based on these data and larval performance  
278 results, it can be hypothesized that dietary ARA level has an effect on larval survival, and  
279 dietary EPA/ARA ratio has an effect on larval growth. The balance between dietary  
280 content of EPA and ARA has serious consequences on the production of eicosanoids, as  
281 it has been cited that dietary supplementation of EPA can be beneficial by reducing the  
282 excess eicosanoid production from ARA, involved in the high incidences of  
283 cardiovascular and inflammatory conditions and some cancers in mammals (Tocher,  
284 2003). Arachidonic acid is related to the stress response in fish because it is the precursor  
285 of PGE<sub>2</sub>, a prostaglandin that regulates the cortisol response in mammals (Lands, 1991)  
286 and possibly the homologous hypothalamus-pituitary-interrenal (HPI) axis in fish (Gupta  
287 et al., 1985). The HPI axis is involved in the appetite-suppressing effects of stress,  
288 regulating food intake in fish (Bernier & Peter, 2001).

289 A strong relationship between dietary FA and the FA composition of larvae was  
290 found (Table 5), which is consistent with other studies on larval fish (Bransden et al 2004,  
291 Copeman et al 2002, Furuita et al 1999, Mourente et al 1999b, Van Anholt et al 2004,  
292 Villalta et al 2005, Willey et al 2003). Increasing dietary ARA concentration resulted in  
293 a concomitant increase in tissue ARA. Tissue concentration of initial and control larvae  
294 showed about 7% EPA content that was not conserved when the larvae were fed ARA-  
295 enriched live prey. This displacement of tissue EPA by ARA has been attributed to the  
296 competitive interaction between these two FA (Bell et al., 1995).

#### 297 Experiment for Eicosapentaenoic Acid Dietary Requirements

298 No significant differences between groups were detected in terms of survival or  
299 growth (Table 4), but a higher, although not statistically significant, survival rate was

300 obtained in the larvae fed EPA-H enriched rotifers. The FA composition of the larvae  
301 (Table 5) did not match that of the prey, especially in the case of EPA-H fed fish. Two  
302 hypotheses can explain the lower EPA levels found in the larvae fed EPA-H enriched  
303 rotifers: 1) an enrichment loss of the rotifer that leads to larvae feeding prey with lower  
304 EPA content than that found in newly enriched ones, or 2) the catabolism of EPA by the  
305 larvae, suggested by the increasing levels of docosapentaenoic acid (DPA, 22:5n-3) and  
306 DHA in larvae tissues. The first hypothesis is fairly unlikely since it requires a  
307 differential, specific enrichment loss for this EFA and level, because the same decrease  
308 is not detected in all the other larvae groups fed with enriched rotifers. The second  
309 hypothesis cannot be validated with the presented results and further investigation is  
310 needed to clarify this point. Several authors (Castell et al., 1994; Bransden et al., 2004)  
311 suggested the relationships between dietary levels and tissue FA accumulation and  
312 depletion as a good tool in assessing nutritional deficiencies in marine fish larvae. In this  
313 sense, higher proportions of ARA and DHA in the larvae than in the diet indicate a  
314 preferential retention of these FA and suggest their dietary essentiality; high levels of  
315 DPA may represent chain elongation as Bransden et al. (2004) observed in striped  
316 trumpeter larvae. All treatments except for the EPA-L group had lower amounts of EPA  
317 in the larvae than in the diet; this could indicate that EPA is not needed at the high  
318 concentrations found in the EPA-M and EPA-H enrichment (8.8 and 16.7%,  
319 respectively). Dietary levels similar to EPA-L diet or slightly higher might be closer to  
320 the specific larval requirement.

321 Trends detected during early larval stages that are not significantly different can  
322 become significantly different in later developmental stages; biomass increase in time and  
323 reproducible high survival rates after metamorphosis are among the indexes used to

324 evaluate larval rearing techniques and FA requirements. Total lipid content can also be  
325 considered as an indicator of good larval performance, since the larvae that show active  
326 feeding behavior optimize the energy needed for hunting and the nutrients obtained from  
327 the diet, as FA. Consequently, active feeding larvae accumulate lipid reserves instead of  
328 expending them in compensating growth depensation, dietary imbalances or other types  
329 of environmental/biochemical stress later in development. From this point of view, larvae  
330 fed EPA-H enriched rotifers showed a better condition than those fed rotifers enriched  
331 with lower levels of EPA. The results obtained with EPA dietary requirements should  
332 take into account DHA and ARA dietary levels, due to the role that both EFA play in the  
333 composition of cell membranes, especially those of the neural system. The ratio  
334 DHA/EPA of EPA-M and EPA-H enriched rotifers is lower (0.7 and 0.5, respectively)  
335 than the ratio obtained in 15 dph larvae fed with this type of rotifer (1.7 and 1.2,  
336 respectively) with the differences observed being a consequence of the accumulation of  
337 DHA by the larvae and/or the consumption of EPA. Larvae fed EPA-L enriched rotifers  
338 showed a DHA/EPA ratio similar to that found in the diet (2.3 and 2.5, respectively) and  
339 in common dentex larvae of the same age reared under different culturing conditions and  
340 fed live prey enriched with commercial products (2.0; Giménez et al., 2008); these data  
341 might indicate a larval requirement for EPA close to the EPA-L diet. Chain elongation of  
342 EPA observed in other larvae such as Morone saxatilis (Harel et al., 2001) and Latris  
343 lineata (Bransden et al., 2004) might also be considered. Bransden et al. (2004) explained  
344 the accumulation of DPA as a consequence of EPA elongation and suggested DPA as an  
345 early indicator of DHA deficiency. Larvae fed EPA-H enriched rotifers also fed the  
346 highest EPA/ARA level, which can have consequences in the production of eicosanoids



347 by the larvae, making it impossible to separate both effects, the positive effect of  
348 EPA/ARA ratio and EPA dietary content, on larval performance.

#### 349 Comparison of Both Experiments and with Published Data

350 Common dentex larval survival is one of the main bottlenecks for the commercial  
351 culture of this finfish species (Giménez 2008). Survival rates during the first days of larval  
352 development vary dramatically depending on the zootechnical parameters (Giménez and  
353 Estévez 2008a) and depend on egg quality (Giménez et al 2006). Survival rates in  
354 experimental conditions below 5% are quite common, consequently, the results obtained  
355 in the present experiments, in terms of survival, are low compared to other finfish larvae  
356 but fall within the normal for common dentex larvae.

357 Larvae used in both experiments had the same genetic background because they  
358 were obtained from the same egg batch. Larvae in the EPA experiment were smaller than  
359 those in the ARA experiment, indicating the effect of different rearing tanks (100-L tanks  
360 vs 35-L baskets) on growth. Taking into account the overall larval performance (Tables  
361 4 and 5) and the FA composition of the delivered rotifers (Table 3), the best ARA and  
362 EPA dietary levels for premetamorphic larvae of common dentex (i.e. from 3 to 15 dph)  
363 are less than 2% ARA, 5 – 15% EPA and keeping the EPA/ARA ratio around 7.

364 Other studies on the effects of different dietary levels of ARA on marine fish larval  
365 performance obtained opposed effects, depending on ARA levels and fish species.  
366 Positive effects of supplementing with ARA before exposure to stressors were found by  
367 Van Anholt et al (2004) on growth and stress responses of Sparus aurata larvae. Willey  
368 et al (2003) determined the ARA dietary requirement for Paralichthys dentatus larvae  
369 (6%) and Ishizaki et al (1998) for Seriola quinqueradiata larvae (<4%). Oppositely,  
370 negative effects on pigmentation with higher ARA levels were described by Lund et al

371 (2007) for Solea solea (>10%) and Villalta et al (2005) for Solea senegalensis (14.8%); it  
372 must be noted that the levels used by these authors are higher than that used in the present  
373 experiment. Bransden et al (2004) did not find any effect of dietary ARA levels on Latris  
374 lineata growth or swimbladder inflation.

375 The requirements of common dentex larvae for dietary EPA are close to those  
376 found in fish larvae from temperate water such as Pseudocaranx dentex (>3.1%; Takeuchi  
377 et al., 1996) and Limanda ferruginea (3.5%; Copeman et al., 2002). Common dentex EPA  
378 requirements were higher than those of the temperate species Paralichthys olivaceus (1%;  
379 Furuita et al., 1999) and lower than that of the warm water species Sparidentex hasta  
380 (19.3%; Abu-Rezq et al., 2002).

381 These data suggest that the effect of dietary ARA and EPA on larval performance  
382 is complex, species specific, and potentially related to larval age and stage of  
383 development. Furthermore, it is possible that when evaluating the relationship between  
384 dietary ARA and the physiological performance in marine fish, their natural distribution  
385 (temperate or tropical, pelagic or benthic) must be considered.

### 386 **Conclusions**

387 Dietary requirements of ARA and EPA in premetamorphic larvae of common  
388 dentex are less than 2% ARA, 5 – 15% EPA and an EPA/ARA ratio of 7. Dietary ARA  
389 requirements of common dentex larvae are low, compared to other marine finfish species.  
390 EPA requirement during this period of larval development does not seem to be as critical  
391 as previously supposed, although the results indicate a higher EPA requirement later in  
392 development, in agreement with the lipid composition of common dentex larvae during  
393 its development.

394

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395

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401 larval rearing and sampling, and to Gordon Bell, Jim Henderson and the staff of the  
402 Laboratory of Fish Nutrition of University of Stirling where lipid samples were analyzed.

403

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528 TABLES

529

530 TABLE 1. Formulation per 100g of emulsion; amount of each compound in grams.  
531 Commercial trademarks used: Vevodar<sup>TM</sup> (DSM IP ASSETS B.V., Netherlands),  
532 Neuromins<sup>TM</sup> (Martek Biosciences Corp., USA), EPA500<sup>TM</sup> (Croda International Plc.,  
533 UK). Olive oil was from the variety cornicabra. All the compounds were mixed in 42  
534 grams of distilled water at 50°C.

535

536 TABLE 2. Emulsion compositions (%). Superscripts indicate significant differences ( $P <$   
537  $0.05$ , Tukey's test,  $N=3$ ). SFA: saturated fatty acids. MUFA: monounsaturated fatty acids  
538 (including 20:1, 22:1, 24:1). PUFA: polyunsaturated fatty acids. Total n-6 includes 18:3n-  
539 6, 20:3n-6, 20:5n-6, 22:4n-6 and 22:5n-6. Total n-3 includes 18:4n-3, 20:3n-3, 20:4n-3  
540 and 22:5n-3. Note that total lipids and total FA units are  $\text{mg g}^{-1}$  DW.

541

542 TABLE 3. Results of fatty acid analysis (% , mean  $\pm$  SD) of rotifers. Superscripts denote  
543 significant differences ( $P < 0.05$ , Tukey's test,  $N = 3$ ) between groups of the same set-up.  
544 SFA: saturated fatty acids. MUFA: monounsaturated fatty acids (including 20:1, 22:1,  
545 24:1). PUFA: polyunsaturated fatty acids. Total n-6 includes 18:3n-6, 20:3n-6, 20:5n-6,  
546 22:4n-6 and 22:5n-6. Total n-3 includes 18:4n-3, 20:3n-3, 20:4n-3 and 22:5n-3. Note that  
547 total lipids and total FA units are  $\text{mg g}^{-1}$  DW.

548

549 TABLE 4. Survival and growth results. Superscripts denote significant differences  
550 between groups from the same experimental set up (ARA or EPA).

551

552 TABLE 5. Larvae composition of experiment on ARA and EPA requirements.  
553 Superscripts indicate significant differences ( $P < 0.05$ , Tukey's test) among 15 dph larvae  
554 of the same experimental set-up.

555 TABLE 1. Formulation per 100g of emulsion; amount of each compound in grams. Commercial trademarks used: Vevodar™ (DSM IP ASSETS  
 556 B.V., Netherlands), Neuromins™ (Martek BBiosciences Corp., USA), EPA500™ (Croda International Plc., UK). Olive oil was from the variety  
 557 cornicabra. All the compounds were mixed in 42 grams of distilled water at 50°C.

558

	ARA-Low	ARA-Medium	ARA-High	EPA-Low	EPA-Medium	EPA-High
Vevodar™	0.00	8.70	17.40	3.13	1.51	0.00
Neuromins™	29.00	29.00	29.00	7.66	3.83	0.00
EPA500™	0.00	0.00	0.00	17.57	35.15	52.78
Olive oil	20.59	12.88	5.22	24.42	12.3	0.00
Corn oil	2.03	1.04	0.00	0.00	0.00	0.00
Soy lecithin	4.10	4.10	4.10	4.06	4.06	4.06
α-tocophenol	2.32	2.32	2.32	1.16	1.16	1.16

559

560

561 TABLE 2. Emulsion compositions (%). Superscripts indicate significant differences ( $P < 0.05$ , Tukey's test,  $N=3$ ). SFA: saturated fatty acids.  
 562 MUFA: monounsaturated fatty acids (including 20:1, 22:1, 24:1). PUFA: polyunsaturated fatty acids. Total n-6 includes 18:3n-6, 20:3n-6, 20:5n-  
 563 6, 22:4n-6 and 22:5n-6. Total n-3 includes 18:4n-3, 20:3n-3, 20:4n-3 and 22:5n-3. Note that total lipids and total FA units are  $\text{mg g}^{-1}$  DW.

	Easy DHA Selco®	ARA-Low	ARA-Medium	ARA-High	EPA-Low	EPA-Medium	EPA-High
Total lipids/DW ( $\text{mg g}^{-1}$ )	120.42 ± 4.69 <sup>b</sup>	841.29 ± 93.86 <sup>a</sup>	886.20 ± 131.42 <sup>a</sup>	896.15 ± 100.53 <sup>a</sup>	621.7 ± 130.7	486.6 ± 89.9	472.2 ± 53.2
FAMES/DW ( $\text{mg g}^{-1}$ )	57.70 ± 0.73 <sup>b</sup>	597.39 ± 58.90 <sup>a</sup>	624.19 ± 62.17 <sup>a</sup>	658.45 ± 68.49 <sup>a</sup>	466.9 ± 68.7	357.8 ± 83.1	331.9 ± 46.5
16:0	12.1 ± 0.6	13.2 ± 0.6	13.2 ± 0.1	12.4 ± 0.9	9.5 ± 0.5 <sup>a</sup>	8.4 ± 0.2 <sup>ab</sup>	6.8 ± 0.4 <sup>b</sup>
18:0	2.7 ± 0.3 <sup>ab</sup>	2.2 ± 0.1 <sup>c</sup>	2.3 ± 0.0 <sup>bc</sup>	2.9 ± 0.2 <sup>a</sup>	3.1 ± 0.1 <sup>a</sup>	2.7 ± 0.1 <sup>a</sup>	2.0 ± 0.1 <sup>b</sup>
SFA	17.7 ± 1.2 <sup>b</sup>	22.7 ± 0.6 <sup>a</sup>	23.6 ± 0.1 <sup>a</sup>	23.0 ± 1.5 <sup>a</sup>	15.1 ± 0.5 <sup>a</sup>	13.5 ± 0.5 <sup>ab</sup>	11.2 ± 0.3 <sup>b</sup>
16:1	7.2 ± 0.6 <sup>a</sup>	1.2 ± 0.3 <sup>c</sup>	1.4 ± 0.2 <sup>c</sup>	3.1 ± 0.2 <sup>b</sup>	1.6 ± 0.5	1.5 ± 0.8	0.9 ± 0.1
18:1	14.8 ± 1.2 <sup>b</sup>	40.8 ± 3.6 <sup>a</sup>	40.6 ± 0.3 <sup>a</sup>	35.2 ± 1.0 <sup>a</sup>	58.4 ± 1.7 <sup>a</sup>	45.8 ± 1.0 <sup>b</sup>	30.9 ± 0.7 <sup>c</sup>
MUFA	28.3 ± 1.9 <sup>b</sup>	42.5 ± 3.3 <sup>a</sup>	42.2 ± 0.1 <sup>a</sup>	38.5 ± 1.3 <sup>a</sup>	60.6 ± 1.3 <sup>a</sup>	48.4 ± 0.9 <sup>b</sup>	33.5 ± 0.8 <sup>c</sup>
18:2n-6	9.0 ± 1.4	10.0 ± 4.7	6.9 ± 0.3	6.8 ± 1.1	8.6 ± 1.4	8.9 ± 1.3	7.6 ± 1.7
20:4n-6	0.8 ± 0.1 <sup>d</sup>	1.6 ± 0.1 <sup>c</sup>	3.3 ± 0.0 <sup>b</sup>	7.6 ± 0.4 <sup>a</sup>	3.5 ± 0.1	3.4 ± 0.1	3.4 ± 0.5
Total n-6	10.7 ± 1.5	11.7 ± 4.5	10.5 ± 0.3	15.0 ± 0.7	12.4 ± 1.3	12.6 ± 1.2	11.2 ± 1.6
18:3n-3	21.8 ± 2.1 <sup>a</sup>	0.5 ± 0.4 <sup>b</sup>	0.6 ± 0.0 <sup>b</sup>	0.4 ± 0.1 <sup>b</sup>	1.1 ± 0.2	1.4 ± 0.2	1.4 ± 0.2

20:5 <i>n</i> -3	5.3 ± 0.4 <sup>a</sup>	0.4 ± 0.2 <sup>b</sup>	0.3 ± 0.1 <sup>b</sup>	0.7 ± 0.5 <sup>b</sup>	2.6 ± 0.1 <sup>c</sup>	13.3 ± 0.8 <sup>b</sup>	29.4 ± 1.1 <sup>a</sup>
22:5 <i>n</i> -3	1.1 ± 0.4 <sup>a</sup>	0.1 ± 0.1 <sup>b</sup>	0.1 ± 0.0 <sup>b</sup>	0.1 ± 0.0 <sup>b</sup>	0.1 ± 0.0 <sup>c</sup>	0.3 ± 0.0 <sup>b</sup>	0.8 ± 0.0 <sup>a</sup>
22:6 <i>n</i> -3	1.2 ± 0.1 <sup>b</sup>	21.5 ± 2.4 <sup>a</sup>	22.7 ± 0.3 <sup>a</sup>	22.2 ± 1.6 <sup>a</sup>	7.7 ± 0.4	8.5 ± 0.7	8.6 ± 0.2
Total <i>n</i> -3	42.4 ± 4.1 <sup>a</sup>	23.2 ± 1.8 <sup>b</sup>	23.7 ± 0.3 <sup>b</sup>	23.5 ± 1.0 <sup>b</sup>	11.9 ± 0.3 <sup>c</sup>	25.5 ± 1.1 <sup>b</sup>	47.1 ± 1.2 <sup>a</sup>
Total PUFA	53.1 ± 2.6 <sup>a</sup>	34.8 ± 3.2 <sup>b</sup>	34.2 ± 0.2 <sup>b</sup>	38.5 ± 0.3 <sup>b</sup>	24.3 ± 1.1 <sup>c</sup>	38.1 ± 0.9 <sup>b</sup>	55.3 ± 3.2 <sup>a</sup>
<i>n</i> -3/ <i>n</i> -6	4.0 ± 0.9	2.2 ± 0.8	2.3 ± 0.1	1.6 ± 0.1	1.0 ± 0.1 <sup>c</sup>	2.0 ± 0.3 <sup>b</sup>	5.9 ± 1.3 <sup>a</sup>
DHA/EPA	0.2 ± 0.0 <sup>c</sup>	57.6 ± 28.6 <sup>b</sup>	85.2 ± 3.3 <sup>a</sup>	58.4 ± 10.4 <sup>b</sup>	3.0 ± 0.1 <sup>a</sup>	0.6 ± 0.0 <sup>b</sup>	0.3 ± 0.0 <sup>c</sup>
DHA/ARA	1.6 ± 0.0 <sup>d</sup>	13.3 ± 0.3 <sup>a</sup>	6.7 ± 0.0 <sup>b</sup>	2.9 ± 0.1 <sup>c</sup>	2.2 ± 0.0 <sup>b</sup>	2.5 ± 0.1 <sup>b</sup>	32.5 ± 1.1 <sup>a</sup>
EPA/ARA	7.0 ± 1.0 <sup>a</sup>	0.3 ± 0.1 <sup>b</sup>	0.1 ± 0.0 <sup>b</sup>	0.1 ± 0.0 <sup>b</sup>	0.7 ± 0.0 <sup>c</sup>	4.0 ± 0.1 <sup>b</sup>	110.8 ± 2.3 <sup>a</sup>

565 TABLE 3. Results of fatty acid analysis (% , mean  $\pm$  SD) of rotifers. Superscripts denote significant differences ( $P < 0.05$ , Tukey's test,  $N = 3$ )  
 566 between groups of the same set-up. SFA: saturated fatty acids. MUFA: monounsaturated fatty acids (including 20:1, 22:1, 24:1). PUFA:  
 567 polyunsaturated fatty acids. Total n-6 includes 18:3n-6, 20:3n-6, 20:5n-6, 22:4n-6 and 22:5n-6. Total n-3 includes 18:4n-3, 20:3n-3, 20:4n-3 and  
 568 22:5n-3. Note that total lipids and total FA units are  $\text{mg g}^{-1}$  DW.

	<u>ARA set-up</u>					<u>EPA set-up</u>			
	Non enriched	Easy DHA Selco®	ARA-Low	ARA- Medium	ARA-High	Non enriched	EPA-Low	EPA-Medium	EPA-High
Total lipids/DW ( $\text{mg g}^{-1}$ )	71.0 $\pm$ 9.3 <sup>c</sup>	122.7 $\pm$ 29.1 <sup>bc</sup>	162.4 $\pm$ 52.2 <sup>ab</sup>	195.5 $\pm$ 30.7 <sup>a</sup>	155.4 $\pm$ 12.3 <sup>abc</sup>	72.4 $\pm$ 6.8 <sup>b</sup>	117.3 $\pm$ 5.6 <sup>ab</sup>	123.5 $\pm$ 14.6 <sup>ab</sup>	240.3 $\pm$ 0.2 <sup>a</sup>
FAME/DW ( $\text{mg g}^{-1}$ )	28.7 $\pm$ 1.3 <sup>b</sup>	74.9 $\pm$ 18.4 <sup>b</sup>	105.5 $\pm$ 24.5 <sup>a</sup>	132.9 $\pm$ 18.7 <sup>a</sup>	110.2 $\pm$ 15.6 <sup>a</sup>	19.9 $\pm$ 1.4 <sup>b</sup>	48.5 $\pm$ 4.9 <sup>b</sup>	52.3 $\pm$ 17.6 <sup>ab</sup>	96.4 $\pm$ 1.8 <sup>a</sup>
16:0	14.4 $\pm$ 0.2	15.0 $\pm$ 0.4	14.1 $\pm$ 0.6	14.8 $\pm$ 0.5	15.0 $\pm$ 0.2	14.4 $\pm$ 0.2 <sup>a</sup>	12.3 $\pm$ 0.1 <sup>b</sup>	11.9 $\pm$ 0.1 <sup>b</sup>	10.9 $\pm$ 1.1 <sup>b</sup>
18:0	3.4 $\pm$ 1.8	4.5 $\pm$ 0.1	2.7 $\pm$ 0.1	3.0 $\pm$ 0.1	3.5 $\pm$ 0.2	3.4 $\pm$ 1.8	4.4 $\pm$ 0.6	4.8 $\pm$ 0.9	3.4 $\pm$ 0.4
SFA	20.2 $\pm$ 1.8 <sup>b</sup>	26.1 $\pm$ 0.9 <sup>a</sup>	24.1 $\pm$ 1.0 <sup>a</sup>	25.6 $\pm$ 0.8 <sup>a</sup>	26.2 $\pm$ 0.2 <sup>a</sup>	20.2 $\pm$ 1.8	20.6 $\pm$ 0.8	20.5 $\pm$ 0.8	18.2 $\pm$ 2.4
16:1	7.5 $\pm$ 0.1 <sup>c</sup>	16.1 $\pm$ 0.6 <sup>a</sup>	1.6 $\pm$ 0.0 <sup>b</sup>	1.6 $\pm$ 0.0 <sup>b</sup>	1.6 $\pm$ 0.1 <sup>b</sup>	7.5 $\pm$ 0.1 <sup>a</sup>	2.5 $\pm$ 0.5 <sup>b</sup>	2.4 $\pm$ 0.5 <sup>b</sup>	2.5 $\pm$ 0.3 <sup>b</sup>
18:1	12.2 $\pm$ 0.0 <sup>d</sup>	25.6 $\pm$ 1.0 <sup>c</sup>	39.3 $\pm$ 0.6 <sup>a</sup>	37.8 $\pm$ 0.2 <sup>a</sup>	32.7 $\pm$ 0.8 <sup>b</sup>	12.2 $\pm$ 0.0 <sup>d</sup>	45.9 $\pm$ 2.2 <sup>a</sup>	37.9 $\pm$ 2.0 <sup>b</sup>	32.1 $\pm$ 2.9 <sup>c</sup>
MUFA	27.5 $\pm$ 0.1 <sup>d</sup>	51.0 $\pm$ 1.4 <sup>a</sup>	43.2 $\pm$ 0.5 <sup>b</sup>	42.1 $\pm$ 0.6 <sup>b</sup>	36.6 $\pm$ 0.0 <sup>c</sup>	27.5 $\pm$ 0.1 <sup>d</sup>	51.3 $\pm$ 1.4 <sup>a</sup>	44.8 $\pm$ 2.3 <sup>b</sup>	37.7 $\pm$ 1.8 <sup>c</sup>
18:2n-6	11.5 $\pm$ 0.4 <sup>a</sup>	2.7 $\pm$ 0.2 <sup>c</sup>	9.0 $\pm$ 0.1 <sup>b</sup>	8.3 $\pm$ 0.3 <sup>b</sup>	8.2 $\pm$ 0.7 <sup>b</sup>	11.5 $\pm$ 0.4 <sup>a</sup>	7.0 $\pm$ 0.4 <sup>b</sup>	6.7 $\pm$ 0.5 <sup>bc</sup>	6.0 $\pm$ 0.1 <sup>c</sup>

20:4 <i>n</i> -6	1.6 ± 0.1 <sup>c</sup>	0.6 ± 0.1 <sup>d</sup>	1.6 ± 0.1 <sup>c</sup>	2.8 ± 0.2 <sup>b</sup>	6.2 ± 0.4 <sup>a</sup>	1.6 ± 0.1 <sup>b</sup>	3.0 ± 0.1 <sup>a</sup>	2.9 ± 0.2 <sup>a</sup>	2.4 ± 0.3 <sup>a</sup>
Total <i>n</i> -6	15.5 ± 0.5 <sup>a</sup>	4.3 ± 0.3 <sup>c</sup>	11.2 ± 0.2 <sup>b</sup>	11.9 ± 0.4 <sup>b</sup>	15.7 ± 0.4 <sup>a</sup>	15.5 ± 0.5 <sup>a</sup>	10.9 ± 0.2 <sup>b</sup>	10.6 ± 0.7 <sup>b</sup>	9.7 ± 0.7 <sup>b</sup>
18:3 <i>n</i> -3	17.7 ± 0.7 <sup>a</sup>	3.6 ± 0.4 <sup>b</sup>	3.1 ± 0.1 <sup>b</sup>	2.8 ± 0.2 <sup>b</sup>	2.8 ± 0.3 <sup>b</sup>	17.7 ± 0.7 <sup>a</sup>	5.0 ± 0.5 <sup>b</sup>	4.5 ± 0.2 <sup>b</sup>	4.0 ± 1.2 <sup>b</sup>
20:5 <i>n</i> -3	3.5 ± 0.2 <sup>b</sup>	4.8 ± 0.3 <sup>a</sup>	0.6 ± 0.1 <sup>c</sup>	0.6 ± 0.1 <sup>c</sup>	0.6 ± 0.2 <sup>c</sup>	3.5 ± 0.2 <sup>c</sup>	2.6 ± 0.1 <sup>c</sup>	8.8 ± 0.6 <sup>b</sup>	16.7 ± 2.2 <sup>a</sup>
22:5 <i>n</i> -3	1.8 ± 0.1 <sup>a</sup>	1.6 ± 0.1 <sup>b</sup>	0.5 ± 0.1 <sup>c</sup>	0.5 ± 0.1 <sup>c</sup>	0.4 ± 0.1 <sup>c</sup>	1.8 ± 0.1 <sup>a</sup>	0.5 ± 0.1 <sup>c</sup>	0.7 ± 0.1 <sup>bc</sup>	0.8 ± 0.1 <sup>b</sup>
22:6 <i>n</i> -3	0.9 ± 0.3 <sup>b</sup>	3.4 ± 0.7 <sup>b</sup>	15.6 ± 1.2 <sup>a</sup>	15.0 ± 1.0 <sup>a</sup>	16.2 ± 1.5 <sup>a</sup>	0.9 ± 0.3 <sup>b</sup>	6.0 ± 0.7 <sup>a</sup>	6.3 ± 0.5 <sup>a</sup>	8.0 ± 1.8 <sup>a</sup>
Total <i>n</i> -3	33.8 ± 1.0 <sup>a</sup>	17.4 ± 0.9 <sup>c</sup>	21.1 ± 1.4 <sup>b</sup>	20.0 ± 1.1 <sup>b</sup>	21.2 ± 0.8 <sup>b</sup>	33.8 ± 1.0 <sup>a</sup>	16.7 ± 0.6 <sup>c</sup>	23.5 ± 0.9 <sup>b</sup>	33.6 ± 2.8 <sup>a</sup>
Total PUFA	49.2 ± 1.5 <sup>a</sup>	21.6 ± 0.8 <sup>d</sup>	32.3 ± 1.6 <sup>c</sup>	31.9 ± 1.4 <sup>c</sup>	36.9 ± 0.4 <sup>b</sup>	49.2 ± 1.5 <sup>a</sup>	27.6 ± 0.8 <sup>d</sup>	34.1 ± 1.6 <sup>c</sup>	43.2 ± 3.5 <sup>b</sup>
<i>n</i> -3/ <i>n</i> -6	2.2 ± 0.0 <sup>b</sup>	4.1 ± 0.4 <sup>a</sup>	1.9 ± 0.1 <sup>bc</sup>	1.7 ± 0.1 <sup>bc</sup>	1.3 ± 0.0 <sup>c</sup>	2.2 ± 0.0 <sup>b</sup>	1.5 ± 0.0 <sup>c</sup>	2.2 ± 0.1 <sup>b</sup>	3.5 ± 0.2 <sup>a</sup>
DHA/EPA	0.3 ± 0.1 <sup>b</sup>	0.7 ± 0.1 <sup>b</sup>	25.4 ± 2.4 <sup>a</sup>	26.6 ± 6.7 <sup>a</sup>	28.0 ± 11.7 <sup>a</sup>	0.3 ± 0.1 <sup>c</sup>	2.3 ± 0.2 <sup>a</sup>	0.7 ± 0.0 <sup>b</sup>	0.5 ± 0.1 <sup>bc</sup>
DHA/ARA	0.6 ± 0.2 <sup>d</sup>	5.9 ± 1.4 <sup>b</sup>	9.7 ± 0.3 <sup>a</sup>	5.5 ± 0.2 <sup>b</sup>	2.6 ± 0.1 <sup>c</sup>	0.6 ± 0.2 <sup>c</sup>	2.0 ± 0.1 <sup>b</sup>	2.2 ± 0.1 <sup>ab</sup>	3.4 ± 0.9 <sup>a</sup>
EPA/ARA	2.1 ± 0.1 <sup>b</sup>	8.6 ± 1.4 <sup>a</sup>	0.4 ± 0.0 <sup>bc</sup>	0.2 ± 0.1 <sup>c</sup>	0.1 ± 0.0 <sup>bc</sup>	2.1 ± 0.1 <sup>c</sup>	0.9 ± 0.0 <sup>d</sup>	3.1 ± 0.2 <sup>b</sup>	7.0 ± 0.2 <sup>a</sup>



570 TABLE 4. Survival and growth results. Superscripts denote significant differences  
 571 between groups from the same experimental set up (ARA or EPA).

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	<u>Survival (%)</u>		<u>DW (<math>\mu</math>g)</u>		<u>TL (mm)</u>	
	ARA	EPA	ARA	EPA	ARA	EPA
Initial (3 dph)	-	-	27.7 $\pm$ 1.0	27.7 $\pm$ 1.0 <sup>b</sup>	3.2 $\pm$ 0.1	3.2 $\pm$ 0.1 <sup>b</sup>
Easy DHA Selco® (15 dph)	5.7 $\pm$ 1.9 <sup>a</sup>	-	215.6 $\pm$ 50.2 <sup>a</sup>	-	4.8 $\pm$ 0.8 <sup>a</sup>	-
Low (15 dph)	5.6 $\pm$ 0.2 <sup>a</sup>	2.8 $\pm$ 2.3	150.4 $\pm$ 29.3 <sup>b</sup>	89.1 $\pm$ 26.8 <sup>a</sup>	4.3 $\pm$ 0.5 <sup>b</sup>	4.0 $\pm$ 0.5 <sup>a</sup>
Medium (15 dph)	4.0 $\pm$ 0.6 <sup>ab</sup>	2.6 $\pm$ 2.1	106.1 $\pm$ 49.9 <sup>b</sup>	90.9 $\pm$ 14.1 <sup>a</sup>	4.2 $\pm$ 0.6 <sup>b</sup>	4.1 $\pm$ 0.4 <sup>a</sup>
High (15 dph)	2.3 $\pm$ 0.6 <sup>b</sup>	4.2 $\pm$ 2.0	127.7 $\pm$ 41.7 <sup>b</sup>	88.7 $\pm$ 29.1 <sup>a</sup>	4.3 $\pm$ 0.6 <sup>b</sup>	4.2 $\pm$ 0.5 <sup>a</sup>

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575 TABLE 5. Larvae composition of experiment on ARA and EPA requirements. Superscripts indicate significant differences ( $P < 0.05$ , Tukey's  
 576 test) among 15 dph larvae of the same experimental set-up.

	<u>ARA set-up</u>					<u>EPA set-up</u>			
		Easy DHA	ARA-Low	ARA-	ARA-High		EPA-Low	EPA-	EPA-High
	0 dph	Selco®		Medium		0 dph		Medium	
Total lipids/DW ( $\text{mg g}^{-1}$ )	74.4 ± 8.9	96.0 ± 10.1	118.7 ± 3.3	79.6 ± 3.1	106.8 ± 11.6	68.7 ± 2.9	130.9 ± 0.5 <sup>b</sup>	167.7 ± 1.8 <sup>ab</sup>	178.0 ± 16.8 <sup>a</sup>
FAME/DW ( $\text{mg g}^{-1}$ )	32.9 ± 7.2	31.6 ± 8.1	33.9 ± 1.0	20.2 ± 2.0	30.5 ± 1.1	30.2 ± 4.1	37.0 ± 2.3	47.8 ± 0.1	43.1 ± 2.8
16:0	17.6 ± 0.4	17.2 ± 0.3	15.5 ± 0.7	18.0 ± 0.4	16.6 ± 0.4	17.6 ± 0.4	16.5 ± 0.3	15.8 ± 0.9	17.6 ± 0.3
18:0	6.2 ± 0.2	9.4 ± 0.1	8.8 ± 0.2	9.7 ± 0.2	8.8 ± 0.7	6.2 ± 0.2	8.1 ± 0.2 <sup>b</sup>	8.7 ± 0.1 <sup>b</sup>	9.8 ± 0.3 <sup>a</sup>
SFA	27.2 ± 0.8	29.2 ± 0.2	26.0 ± 0.3	30.6 ± 0.2	27.8 ± 0.9	27.2 ± 0.8	27.0 ± 0.7 <sup>ab</sup>	26.6 ± 0.7 <sup>b</sup>	30.4 ± 1.1 <sup>a</sup>
16:1	7.5 ± 0.2	6.3 ± 0.1	4.7 ± 0.3	5.7 ± 1.3	4.6 ± 0.5	7.5 ± 0.2	5.2 ± 0.2	4.7 ± 0.5	5.6 ± 0.4
18:1	20.2 ± 0.1	19.9 ± 0.8 <sup>b</sup>	24.0 ± 1.2 <sup>a</sup>	20.1 ± 1.4 <sup>ab</sup>	20.7 ± 1.4 <sup>ab</sup>	20.2 ± 0.1	26.7 ± 0.2 <sup>a</sup>	24.6 ± 1.8 <sup>ab</sup>	20.7 ± 1.4 <sup>b</sup>
MUFA	31.0 ± 0.2	30.6 ± 0.8 <sup>ab</sup>	32.8 ± 1.1 <sup>a</sup>	30.7 ± 0.3 <sup>ab</sup>	29.5 ± 1.7 <sup>b</sup>	31.0 ± 0.2	35.3 ± 0.4	33.0 ± 1.5	31.0 ± 2.0
18:2 $n$ -6	8.5 ± 0.0	7.4 ± 0.7	9.7 ± 0.6	9.3 ± 2.7	9.1 ± 1.0	8.5 ± 0.0	9.7 ± 0.2 <sup>a</sup>	8.7 ± 0.5 <sup>ab</sup>	7.1 ± 0.4 <sup>b</sup>
20:4 $n$ -6	1.5 ± 0.1	2.3 ± 0.1 <sup>c</sup>	4.7 ± 0.6 <sup>b</sup>	4.1 ± 0.5 <sup>b</sup>	6.1 ± 0.4 <sup>a</sup>	1.5 ± 0.1	4.4 ± 0.1 <sup>a</sup>	4.1 ± 0.2 <sup>a</sup>	3.1 ± 0.2 <sup>b</sup>

Total <i>n</i> -6	11.0 ± 0.2	11.5 ± 0.5 <sup>b</sup>	15.8 ± 0.1 <sup>a</sup>	14.9 ± 3.2 <sup>ab</sup>	16.8 ± 0.7 <sup>a</sup>	11.0 ± 0.2	15.5 ± 0.2 <sup>a</sup>	13.9 ± 0.2 <sup>a</sup>	11.5 ± 0.9 <sup>b</sup>
18:3 <i>n</i> -3	0.9 ± 0.1	1.7 ± 0.2	1.8 ± 1.0	1.2 ± 0.7	1.4 ± 0.3	0.9 ± 0.1	2.1 ± 0.0 <sup>a</sup>	1.6 ± 0.1 <sup>b</sup>	1.9 ± 0.1 <sup>a</sup>
20:5 <i>n</i> -3	6.7 ± 0.1	7.2 ± 0.2 <sup>a</sup>	2.7 ± 0.2 <sup>b</sup>	2.9 ± 0.4 <sup>b</sup>	2.9 ± 0.2 <sup>b</sup>	6.7 ± 0.1	4.8 ± 0.1 <sup>b</sup>	7.5 ± 0.5 <sup>ab</sup>	9.3 ± 1.5 <sup>a</sup>
22:5 <i>n</i> -3	1.5 ± 0.0	4.1 ± 0.2 <sup>a</sup>	1.1 ± 0.2 <sup>b</sup>	1.1 ± 0.0 <sup>b</sup>	1.1 ± 0.2 <sup>b</sup>	1.5 ± 0.0	1.3 ± 0.0 <sup>b</sup>	1.6 ± 0.1 <sup>ab</sup>	2.1 ± 0.3 <sup>a</sup>
22:6 <i>n</i> -3	20.4 ± 0.4	13.2 ± 1.8	18.3 ± 2.1	17.1 ± 4.2	19.2 ± 2.3	20.4 ± 0.4	12.2 ± 0.1 <sup>ab</sup>	12.6 ± 0.3 <sup>a</sup>	11.5 ± 0.0 <sup>b</sup>
Total <i>n</i> -3	30.9 ± 0.6	28.7 ± 1.4	25.4 ± 1.1	23.8 ± 3.4	26.0 ± 2.2	30.9 ± 0.6	22.2 ± 0.1	26.5 ± 1.1	27.1 ± 2.2
Total PUFA	41.8 ± 0.8	40.2 ± 0.9	41.2 ± 1.0	41.5 ± 4.9	42.7 ± 1.7	41.8 ± 0.8	37.7 ± 0.2	40.4 ± 0.8	38.6 ± 3.1
<i>n</i> -3/ <i>n</i> -6	2.8 ± 0.0	2.5 ± 0.2	1.6 ± 0.1	1.7 ± 0.6	1.6 ± 0.2	2.8 ± 0.0	1.4 ± 0.0 <sup>c</sup>	1.9 ± 0.1 <sup>b</sup>	2.4 ± 0.0 <sup>a</sup>
DHA/EPA	3.1 ± 0.0	1.8 ± 0.3 <sup>b</sup>	6.9 ± 0.8 <sup>a</sup>	5.9 ± 0.7 <sup>ab</sup>	6.7 ± 0.9 <sup>a</sup>	3.1 ± 0.0	2.5 ± 0.1 <sup>a</sup>	1.7 ± 0.1 <sup>b</sup>	1.2 ± 0.2 <sup>b</sup>
DHA/ARA	13.2 ± 0.5	5.8 ± 0.6 <sup>a</sup>	3.9 ± 0.1 <sup>b</sup>	4.3 ± 1.5 <sup>ab</sup>	3.1 ± 0.2 <sup>c</sup>	13.2 ± 0.5	2.8 ± 0.0 <sup>b</sup>	3.1 ± 0.1 <sup>b</sup>	3.8 ± 0.2 <sup>a</sup>
EPA/ARA	4.3 ± 0.2	3.2 ± 0.2 <sup>a</sup>	0.6 ± 0.1 <sup>b</sup>	0.7 ± 0.2 <sup>b</sup>	0.5 ± 0.0 <sup>b</sup>	4.3 ± 0.2	1.1 ± 0.0 <sup>c</sup>	1.8 ± 0.0 <sup>b</sup>	3.0 ± 0.3 <sup>a</sup>

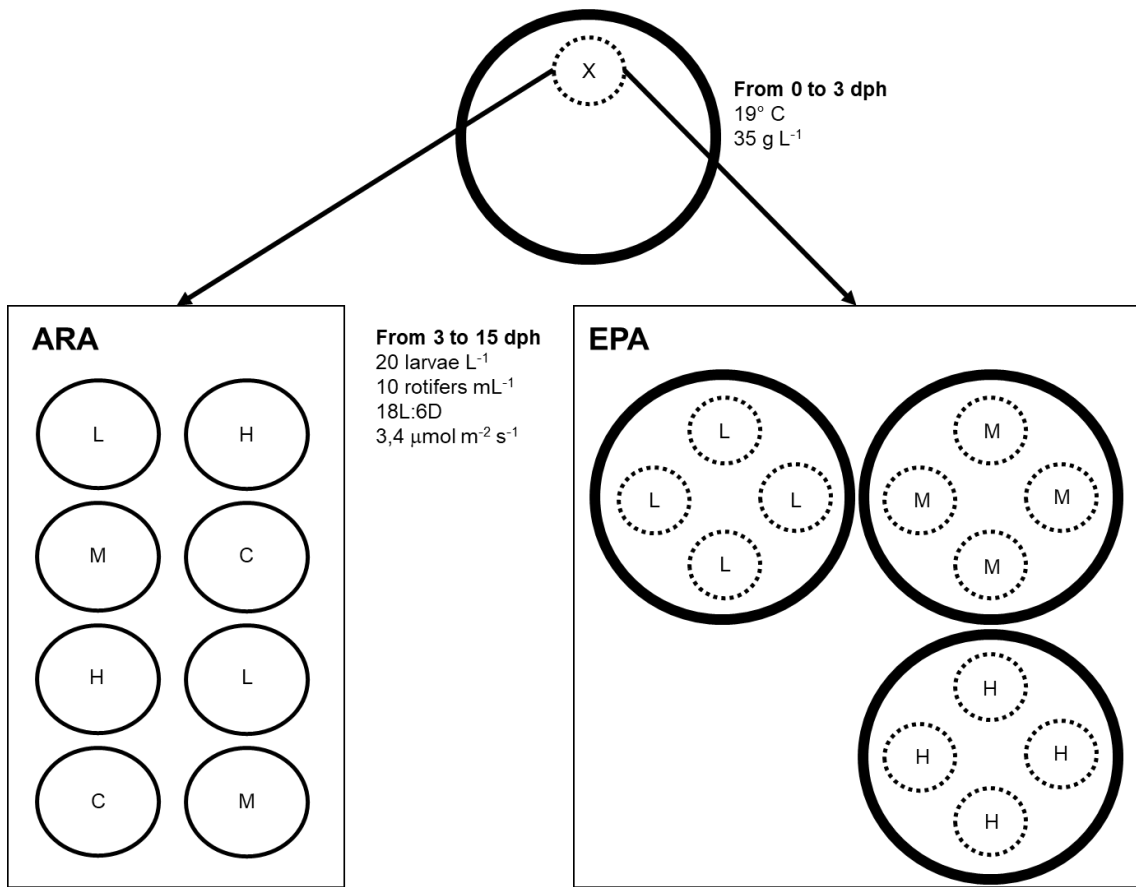
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579 FIGURES

580 FIGURE 1. Graphic summary of the experimental setup. Big thick black circles are 500-  
581 L tanks, medium black circles are 100-L, dashed circles are 35-L baskets. "X" represents  
582 where the eggs were incubated. ARA = arachidonic acid experimental setup. EPA =  
583 eicosapentaenoic acid experimental setup. H = high dietary dose of the tested EFA, M =  
584 medium dietary dose of the tested EFA, L = low dietary dose of the tested EFA, C =  
585 control groups.

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