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

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Abstract

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

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

Introduction

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

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

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

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

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

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

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

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

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

Materials and Methods

Spawning Induction and Egg Quality Assessment

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

provided with an air-lift system and aeration supply ("basket"). This container was immersed in a 500-L black-bottomed tank connected to a recirculation unit equipped with mechanical (up to 1 μm diameter), biological and UV filters, and a temperature controller (Carbó et al., 2002). Larvae hatched after 24 h at 19 ± 1 ºC and 35 g L^{-1} salinity.

The same batch of eggs was used for the two experiments. Larvae of 3 dph were stocked at 20 individuals (ind) mL^{-1}, light conditions 18L:6D and 3.4 μmol m^{-2} s^{-1} irradiance in the water surface, and fed once daily with 10 ind mL^{-1} of enriched Brachionus plicatilis (Giménez & Estévez 2008a; Fig. 1). The experiments finished when larvae were 15 dph. Survival, total length (TL), dry weight (DW) and lipid composition of larvae were measured on larvae of 3 and 15 dph.

Experiment for Arachidonic Acid Dietary Requirements

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

Experiment for Eicosapentaenoic Acid Dietary Requirements

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

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

Emulsion Formulation

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

Three levels of essential fatty acids (expressed in % total fatty acids, %TFA) were defined for the enrichment emulsion: Low, L, below the composition of common dentex larvae in cultured conditions (2% ARA, 3% EPA); Medium, M, close to the composition of Tisbe sp. (4% ARA, 15% EPA) and High, H, two times the composition of Tisbe sp. (8.7% ARA, 30% EPA). Control groups (C) were fed with Easy DHA Selco® (INVE, Belgium) enriched rotifers. The formulation of the emulsions is shown in Table 1: Vevodar™ oil (DSM Food Specialties, Netherlands) was the main source of ARA, Neuromins™ oil (Martek Bioscience Corporation, USA) provided mainly DHA, and EPA500™ 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 α-
158 tocophenol (Sigma Aldrich Co., Germany) was required as antioxidant. The emulsions
159 were stored at 4ºC.

160 Rotifer Enrichment

161 Rotifers (Brachionus plicatilis SM morphotype) were cultured at 20ºC, 33 g L^{-1}
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-µm 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^{-1}, 0.1 g emulsion L^{-1}, during 2 h at 20ºC
169 with continuous light and aeration. After enrichment, rotifers were filtered using a 60-µm
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,

Germany) and image analysis software (AnalySIS Gmbh, Olympus, Germany) to the 0.01 mm. The same individuals were then sacrificed by MS 222 excess, filtered using a 150-μm hand-made mesh, gently rinsed with tap water and distilled water. Excess water was released and dried. Larvae were weighed (Wet Weight, WW), oven-dried at 60º C for 24 h, and weighed again to obtain Dry Weight (DW) to the nearest μg on a Mettler A-20 microbalance (Mettler Toledo, Columbus, OH, USA). Averaged water percentage (%W) was estimated from these data.

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

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

Lipid analysis

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

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

Statistical Analysis

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

Results and Discussion

Fatty Acid Composition of the Delivered Rotifer

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

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

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

When the percentage of a specific EFA is modified in the diet, other important fatty acids vary. Substrates for B-oxidation (SFA and MUFA) and DHA should be also taken into account. In this experiment, there were no differences in the percentage of SFA between ARA diets (only when they were compared to the non enriched rotifer) neither between EPA diets (Table 3). The percentage of MUFA were not different between ARA diets, but did differ in EPA diets (Table 3). Regarding the percentage of DHA, there were no differences between the diets used in the same setup (Table 3), therefore, the differences observed among the experimental groups were not due to the level of this 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±50.2 μg and
265 4.8±0.8mm, average ± SD). Larvae fed rotifers enriched with ARA-L emulsions showed
266 a similar survival rate (5.6%) but lower growth (150.4±29.3 μg and 4.3±0.5 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

ARA levels (1.6%), lower EPA levels (0.6%, similar to all rotifers enriched with ARA emulsions) and lower EPA/ARA ratios (0.4). Based on these data and larval performance results, it can be hypothesized that dietary ARA level has an effect on larval survival, and dietary EPA/ARA ratio has an effect on larval growth. The balance between dietary content of EPA and ARA has serious consequences on the production of eicosanoids, as it has been cited that dietary supplementation of EPA can be beneficial by reducing the excess eicosanoid production from ARA, involved in the high incidences of cardiovascular and inflammatory conditions and some cancers in mammals (Tocher, 2003). Arachidonic acid is related to the stress response in fish because it is the precursor of PGE2, a prostaglandin that regulates the cortisol response in mammals (Lands, 1991) and possibly the homologous hypothalamus-pituitary-interrenal (HPI) axis in fish (Gupta et al., 1985). The HPI axis is involved in the appetite-suppressing effects of stress, regulating food intake in fish (Bernier & Peter, 2001).

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

Experiment for Eicosapentaenoic Acid Dietary Requirements

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

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

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

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

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

Comparison of Both Experiments and with Published Data

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

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

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

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

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

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

Conclusions

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

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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™ (DSM IP ASSETS B.V., Netherlands),
532 Neuromins™ (Martek Biosciences Corp., USA), EPA500™ (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 50ºC.

535

536 TABLE 2. Emulsion compositions (%). Superscripts indicate significant differences ($P < 0.05$, 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 mg g^{-1} DW.

541

542 TABLE 3. Results of fatty acid analysis (%, mean ± 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 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 50ºC.

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 mg g^{-1} DW.

	Easy DHA Selco®	ARA-Low	ARA-Medium	ARA-High	EPA-Low	EPA-Medium	EPA-High
Total lipids/DW (mg g^{-1})	120.42 ± 4.69^{b}	841.29 ± 93.86^{a}	886.20 ± 131.42^{a}	896.15 ± 100.53^{a}	621.7 ± 130.7	486.6 ± 89.9	472.2 ± 53.2
FAMES/DW (mg g^{-1})	57.70 ± 0.73^{b}	597.39 ± 58.90^{a}	624.19 ± 62.17^{a}	658.45 ± 68.49^{a}	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^{a}	8.4 ± 0.2^{ab}	6.8 ± 0.4^{b}
18:0	2.7 ± 0.3^{ab}	2.2 ± 0.1^{c}	2.3 ± 0.0^{bc}	2.9 ± 0.2^{a}	3.1 ± 0.1^{a}	2.7 ± 0.1^{a}	2.0 ± 0.1^{b}
SFA	17.7 ± 1.2^{b}	22.7 ± 0.6^{a}	23.6 ± 0.1^{a}	23.0 ± 1.5^{a}	15.1 ± 0.5^{a}	13.5 ± 0.5^{ab}	11.2 ± 0.3^{b}
16:1	7.2 ± 0.6^{a}	1.2 ± 0.3^{c}	1.4 ± 0.2^{c}	3.1 ± 0.2^{b}	1.6 ± 0.5	1.5 ± 0.8	0.9 ± 0.1
18:1	14.8 ± 1.2^{b}	40.8 ± 3.6^{a}	40.6 ± 0.3^{a}	35.2 ± 1.0^{a}	58.4 ± 1.7^{a}	45.8 ± 1.0^{b}	30.9 ± 0.7^{c}
MUFA	28.3 ± 1.9^{b}	42.5 ± 3.3^{a}	42.2 ± 0.1^{a}	38.5 ± 1.3^{a}	60.6 ± 1.3^{a}	48.4 ± 0.9^{b}	33.5 ± 0.8^{c}
18:2*n*-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
20:4*n*-6	0.8 ± 0.1^{d}	1.6 ± 0.1^{c}	3.3 ± 0.0^{b}	7.6 ± 0.4^{a}	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
18:3*n*-3	21.8 ± 2.1^{a}	0.5 ± 0.4^{b}	0.6 ± 0.0^{b}	0.4 ± 0.1^{b}	$1. 1 \pm 0.2$	1.4 ± 0.2	1.4 ± 0.2

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

564

565 TABLE 3. Results of fatty acid analysis (%, mean ± 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 mg g^{-1} DW.

	ARA set-up					EPA set-up			
	Non enriched	Easy DHA Selco®	ARA-Low	ARA-Medium	ARA-High	Non enriched	EPA-Low	EPA-Medium	EPA-High
Total lipids/DW (mg g^{-1})	71.0 ± 9.3^{c}	122.7 ± 29.1^{bc}	162.4 ± 52.2^{ab}	195.5 ± 30.7^{a}	155.4 ± 12.3^{abc}	72.4 ± 6.8^{b}	117.3 ± 5.6^{ab}	123.5 ± 14.6^{ab}	240.3 ± 0.2^{a}
FAME/DW (mg g^{-1})	28.7 ± 1.3^{b}	74. 9 ± 18.4^{b}	105.5 ± 24.5^{a}	132.9 ± 18.7^{a}	110.2 ± 15.6^{a}	19.9 ± 1.4^{b}	48.5 ± 4.9^{b}	52.3 ± 17.6^{ab}	96.4 ± 1.8^{a}
16:0	14.4 ± 0.2	15.0 ± 0.4	14.1 ± 0.6	14.8 ± 0.5	15.0 ± 0.2	14.4 ± 0.2^{a}	12.3 ± 0.1^{b}	11.9 ± 0.1^{b}	10.9 ± 1.1^{b}
18:0	3.4 ± 1.8	4.5 ± 0.1	2.7 ± 0.1	3.0 ± 0.1	3.5 ± 0.2	3.4 ± 1.8	4.4 ± 0.6	4.8 ± 0.9	3.4 ± 0.4
SFA	20.2 ± 1.8^{b}	26.1 ± 0.9^{a}	24.1 ± 1.0^{a}	25.6 ± 0.8^{a}	26.2 ± 0.2^{a}	20.2 ± 1.8	20.6 ± 0.8	20.5 ± 0.8	18.2 ± 2.4
16:1	7.5 ± 0.1^{c}	16.1 ± 0.6^{a}	1.6 ± 0.0^{b}	1.6 ± 0.0^{b}	1.6 ± 0.1^{b}	7.5 ± 0.1^{a}	2.5 ± 0.5^{b}	2.4 ± 0.5^{b}	2.5 ± 0.3^{b}
18:1	12.2 ± 0.0^{d}	25.6 ± 1.0^{c}	39.3 ± 0.6^{a}	37.8 ± 0.2^{a}	32.7 ± 0.8^{b}	12.2 ± 0.0^{d}	45.9 ± 2.2^{a}	37.9 ± 2.0^{b}	32.1 ± 2.9^{c}
MUFA	27.5 ± 0.1^{d}	51.0 ± 1.4^{a}	43.2 ± 0.5^{b}	42.1 ± 0.6^{b}	36.6 ± 0.0^{c}	27.5 ± 0.1^{d}	51.3 ± 1.4^{a}	44.8 ± 2.3^{b}	37.7 ± 1.8^{c}
18:2*n*-6	11.5 ± 0.4^{a}	2.7 ± 0.2^{c}	9.0 ± 0.1^{b}	8.3 ± 0.3^{b}	8.2 ± 0.7^{b}	11.5 ± 0.4^{a}	7.0 ± 0.4^{b}	6.7 ± 0.5^{bc}	6.0 ± 0.1^{c}

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

569

570 TABLE 4. Survival and growth results. Superscripts denote significant differences
571 between groups from the same experimental set up (ARA or EPA).
572

	Survival (%)		DW (µg)		TL (mm)	
	ARA	EPA	ARA	EPA	ARA	EPA
Initial (3 dph)	-	-	27.7 ± 1.0	27.7 ± 1.0^{b}	3.2 ± 0.1	3.2 ± 0.1^{b}
Easy DHA Selco® (15 dph)	5.7 ± 1.9^{a}	-	215.6 ± 50.2^{a}	-	4.8 ± 0.8^{a}	-
Low (15 dph)	5.6 ± 0.2^{a}	2.8 ± 2.3	150.4 ± 29.3^{b}	89.1 ± 26.8^{a}	4.3 ± 0.5^{b}	4.0 ± 0.5^{a}
Medium (15 dph)	4.0 ± 0.6^{ab}	2.6 ± 2.1	106.1 ± 49.9^{b}	90.9 ± 14.1^{a}	4.2 ± 0.6^{b}	4.1 ± 0.4^{a}
High (15 dph)	2.3 ± 0.6^{b}	4.2 ± 2.0	127.7 ± 41.7^{b}	88.7 ± 29.1^{a}	4.3 ± 0.6^{b}	4.2 ± 0.5^{a}

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574

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.

		ARA set-up					EPA set-up		
	0 dph	Easy DHA Selco®	ARA-Low	ARA-Medium	ARA-High	0 dph	EPA-Low	EPA-Medium	EPA-High
Total lipids/DW (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^{b}	167.7 ± 1.8^{ab}	178.0 ± 16.8^{a}
FAME/DW (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^{b}	8.7 ± 0.1^{b}	9.8 ± 0.3^{a}
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^{ab}	26.6 ± 0.7^{b}	30.4 ± 1.1^{a}
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^{b}	24.0 ± 1.2^{a}	20.1 ± 1.4^{ab}	20.7 ± 1.4^{ab}	20.2 ± 0.1	26.7 ± 0.2^{a}	24.6 ± 1.8^{ab}	20.7 ± 1.4^{b}
MUFA	31.0 ± 0.2	30.6 ± 0.8^{ab}	32.8 ± 1.1^{a}	30.7 ± 0.3^{ab}	29.5 ± 1.7^{b}	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^{a}	8.7 ± 0.5^{ab}	7.1 ± 0.4^{b}
20:4*n*-6	1.5 ± 0.1	2.3 ± 0.1^{c}	4.7 ± 0.6^{b}	4.1 ± 0.5^{b}	6.1 ± 0.4^{a}	1.5 ± 0.1	4.4 ± 0.1^{a}	4.1 ± 0.2^{a}	3.1 ± 0.2^{b}

Total *n*-6	11.0 ± 0.2	11.5 ± 0.5^{b}	15.8 ± 0.1^{a}	14.9 ± 3.2^{ab}	16.8 ± 0.7^{a}	11.0 ± 0.2	15.5 ± 0.2^{a}	13.9 ± 0.2^{a}	11.5 ± 0.9^{b}
18:3*n*-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^{a}	1.6 ± 0.1^{b}	1.9 ± 0.1^{a}
20:5*n*-3	6.7 ± 0.1	7.2 ± 0.2^{a}	2.7 ± 0.2^{b}	2.9 ± 0.4^{b}	2.9 ± 0.2^{b}	6.7 ± 0.1	4.8 ± 0.1^{b}	7.5 ± 0.5^{ab}	9.3 ± 1.5^{a}
22:5*n*-3	1.5 ± 0.0	4.1 ± 0.2^{a}	1.1 ± 0.2^{b}	1.1 ± 0.0^{b}	1.1 ± 0.2^{b}	1.5 ± 0.0	1.3 ± 0.0^{b}	1.6 ± 0.1^{ab}	2.1 ± 0.3^{a}
22:6*n*-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^{ab}	12.6 ± 0.3^{a}	11.5 ± 0.0^{b}
Total *n*-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
n-3/*n*-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^{c}	1.9 ± 0.1^{b}	2.4 ± 0.0^{a}
DHA/EPA	3.1 ± 0.0	1.8 ± 0.3^{b}	6.9 ± 0.8^{a}	5.9 ± 0.7^{ab}	6.7 ± 0.9^{a}	3.1 ± 0.0	2.5 ± 0.1^{a}	1.7 ± 0.1^{b}	1.2 ± 0.2^{b}
DHA/ARA	13.2 ± 0.5	5.8 ± 0.6^{a}	3.9 ± 0.1^{b}	4.3 ± 1.5^{ab}	3.1 ± 0.2^{c}	13.2 ± 0.5	2.8 ± 0.0^{b}	3.1 ± 0.1^{b}	3.8 ± 0.2^{a}
EPA/ARA	4.3 ± 0.2	3.2 ± 0.2^{a}	0.6 ± 0.1^{b}	0.7 ± 0.2^{b}	0.5 ± 0.0^{b}	4.3 ± 0.2	1.1 ± 0.0^{c}	1.8 ± 0.0^{b}	3.0 ± 0.3^{a}

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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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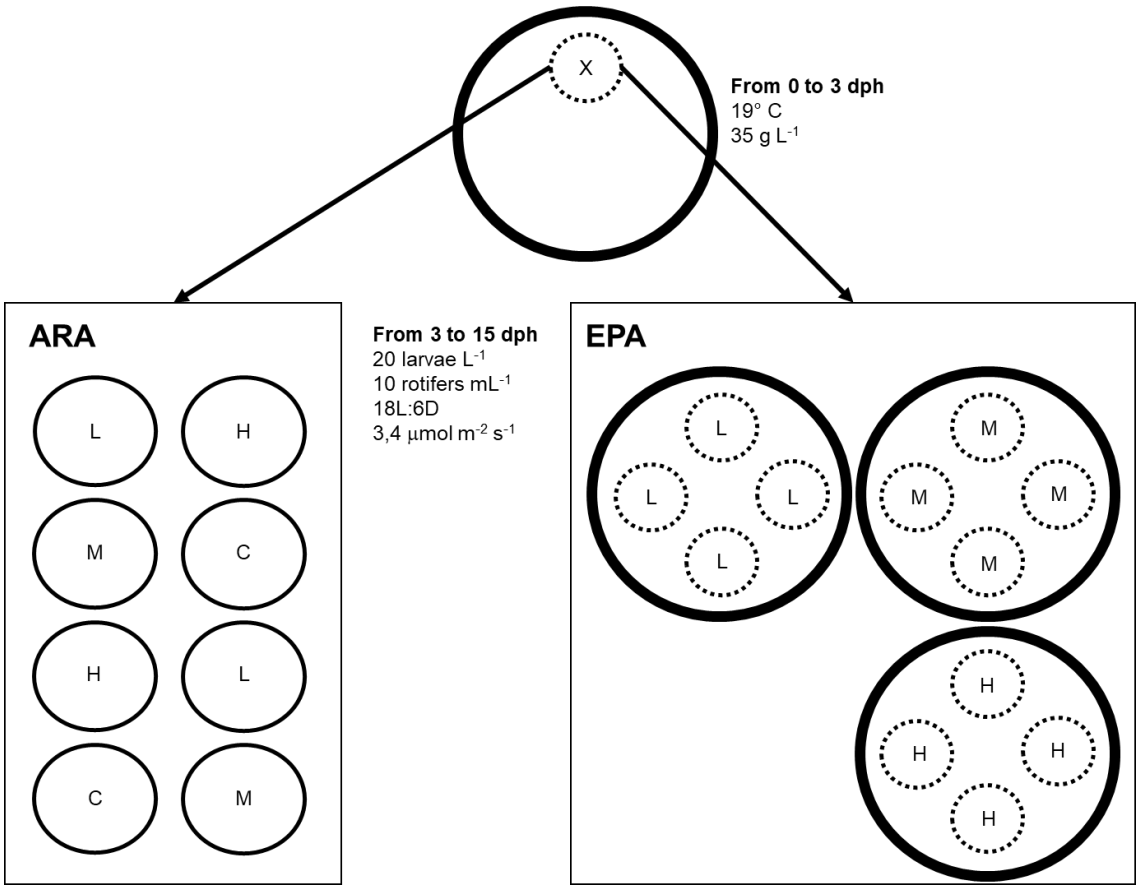

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