Study of the (n-3) highly unsaturated fatty acid requirement and antioxidant status of *Dentex dentex* larvae at the *Artemia* feeding stage

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Abstract

This study was designed to investigate the requirements of *Dentex dentex* larvae for (n-3) highly unsaturated fatty acids (HUFA) at the *Artemia* feeding stage. *Artemia* were enriched using mixtures of ICES Experimental Emulsions ICES 50/0.6/C (500 mg/g (n-3) HUFA, 0.6 DHA/EPA ratio, based on ethyl esters) and ICES 0/-/C (based on coconut oil) and to give five dietary treatments which contained different levels of (n-3) HUFA from 0.72 to 6.23 as dry weight percentage. Optimal growth, as evidenced by total length, individual dry weight, specific growth rate and thermal growth coefficient, was achieved when dietary (n-3) HUFA was 3.97 on a dry weight basis. Larvae fed *Artemia* enriched with apparently super-optimal levels of (n-3) HUFA (5.67-6.23 %) showed significantly lower vitamin E contents and higher malondialdehyde (MDA) levels combined with their eyes having maximum (n-3) HUFA values and DHA/EPA ratios. Poorer performance of larvae was associated with increased dietary and larval MDA and decreased larval vitamin E, indicating increasing oxidation of (n-3) HUFA in *Artemia* and larval utilization of vitamin E with increasing levels of dietary (n-3) HUFA, particularly at supraoptimal levels of enrichment. The activities of antioxidant enzymes in the larvae was generally not greatly affected by the dietary treatments in this study. A balance is required between growth-promoting essential fatty acid (EFA) qualities of (n-3) HUFA and their potentially growth-inhibiting (prooxidant) qualities which must be counter-balanced with adequate dietary antioxidants.

Keywords: (n-3) HUFA; requirements; antioxidant status; Dentex dentex; fish larvae

1. Introduction

The common dentex (*Dentex dentex* L.) is one of the most promising species for marine fish culture in the Mediterranean (Sweetman, 1992; Cataudella et al 1995) and recently some research effort has been directed to the reproduction and larval culture of this species (Pastor el al 1995). Larval production is still a bottleneck in farming marine fish, particularly when attempting a new species whose nutritional requirements are not fully determined. The significance of lipids at early stages of marine fish has been widely studied (Sargent et al., 1989; Sargent et al 1990; Sargent et al 1993b; Watanabe and Kiron, 1994; Sargent, 1995; Wiegand, 1996; Mourente, 1996; Rainuzzo et al 1997) and considerable advances have been made in understanding the polyunsaturated fatty acid nutrition of marine fish larvae (Sargent et al 1997). Moreover, much research has been directed towards determining the (n-3) highly unsaturated fatty acid (HUFA) requirements in larval marine fish (Izquierdo, 1996; Furuita et al 1996a,b). To date, virtually all marine larviculture production systems rely on live feeds, principally the rotifer *Brachionus plicatilis* and nauplii of *Artemia* which are deficient in (n-3) polyunsaturated fatty acids (PUFA), particularly HUFA, and therefore require to be enriched before use (Lavens et al 1995; Coutteau and Mourente 1997; Sargent et al 1997). In consequence, several studies havedealt with the (n-3) HUFA requirements of marine fish fed on live prey, in particular at the *Artemia* feeding stage (Howell and Tzoumas, 1991; Izquierdo et al 1989; 1992; Watanabe and Kiron, 1994; Izquierdo, 1996; Furuita et al 1996a,b).

The present study was designed to evaluate the requirements of *D. dentex* larvae for (n-3) HUFA at the *Artemia* feeding stage by using mixtures of emulsions based on coconut oil and fish oil ethyl esters to give different *Artemia* dietary groups varying in their (n-3) HUFA content. However, one of the potential risks in using *Artemia* enrichment procedures based on fish oil emulsions enriched in (n-3) HUFA is the ease with which these emulsions are prone to autooxidation (McEvoy et al, 1995; Sargent et al 1997). PUFA are readily oxidized by reactive oxygen species to lipid peroxides (Niki, 1987; Porter et al, 1995). So, in consequence, we have also investigated the antioxidant

status and lipid peroxidation processes in the experimental fish by measuring the level of peroxidation products (thiobarbituric acid reactive substances), antioxidant (vitamin E content) and the endogenous antioxidant enzyme system represented by the activities of free radical scavenging enzymes such as catalase, total superoxide dismutase (SOD), Se-dependent and Se-independent glutathione peroxidase (GPX), glutathione-S-transferase (GST) and glutathione reductase (GR). The results were discussed in relation to possible pro-oxidant and antioxidant processes occurring in dentex larvae and the detection of oxidative stress under different dietary treatments.

2. Materials and methods

Broodstock management, egg and larval production

Broodstock maintenance and reproduction, as well as egg and larval production, were performed according to the methodology developed by Pastor et al (1995) at the Estación de Acuicultura, Port d'Andraxt, Palma de Mallorca, Spain. Eggs were hatched in 400 l cylindro-conical tanks in an open circulation system (1-3 water exchange/day) at a density of 100 eggs/l at ambient temperature and 37 ppt salinity. Newly hatched larvae developed at the expense of yolk-sac and oil drop reserves and were then fed from day 4 (when the mouth opens) to day 12 with rotifers *B. plicatilis* strain Bs (Yúfera, 1982) (currently *B. rotundiformis*) cultured with yeast and enriched with DHA Protein Selco (INVE) at a density of 10-15 individual/ml plus greenwater composed of *Nannochloropsis gaditana* and *Isochrysis galbana* (clone T-ISO) at a density of 80000-100000 cells/ml. From day 12 to day 15 *Artemia* nauplii grade AF480 (INVE) at 4-7 individual/ml and from day 15 to day 36 (3 weeks) *Artemia* metanauplii grade EG (INVE), both enriched with the experimental emulsions, were fed.

Experimental emulsions and Artemia enrichment

The emulsions used to enrich *Artemia* were purchased from the Laboratory of Aquaculture & Artemia Refence Center, University of Gent, Gent, Belgium and

consisted of experimental oil emulsions made available within the framework of the International Council for the Exploration of the Sea (ICES) Working Group on the Mass Rearing of Juvenile Fish (ICES 1994). The first emulsion was ICES 0/-/C based on coconut oil, primarily triglycerides, and the second was ICES 50/0.6/C, based on fish oil ethylesters and containing 500 mg (n-3) HUFA/g dry weight and a docosahexaenoic acid/eicosapentaenoic acid (DHA/EPA) ratio of 0.6. The emulsions were mixed in different proportions to get a gradient of (n-3) HUFA for the experimental enrichment emulsions. Emulsion A was prepared by using 100% coconut oil emulsion. Emulsion B was prepared by mixing 95% coconut oil emulsion with 5% of the ethylester fish oil emulsion. Emulsion C contained 80% coconut oil emulsion and 20% fish oil emulsion. Emulsion D contained 40% coconut oil emulsion and 60% fish oil emulsion, and emulsion E was 100% fish oil emulsion. These five treatments were chosen after having tested and analyzed a greater number of mixtures as experimental emulsions. The fatty acid compositions of the emulsions are shown in Table1.

For enrichment, freshly-hatched nauplii of *Artemia* grade EG were placed in an enrichment tank at a density of 200 nauplii·ml^{-1}. The enrichment medium consisted of disinfected seawater at 25ºC. The enrichment emulsions were prepared by mixing a total of 1 g of the ICES Reference Emulsions and 60 ml of distilled water and added in consecutive doses of 350 mg·l^{-1} every 12 h with strong airation to maintain dissolved oxygen levels above 4 mg·l^{-1}. The enriched nauplii were harvested after 24 h, thoroughly rinsed on a sieve under tap water to remove all residual emulsion, and then fed directly to the larvae (Merchie, 1996; Coutteau and Mourente, 1997).

Sample collection

Enriched *Artemia* metanauplii from the different experimental treatments were sampled at 4 time points along the experimental period, on days 15, 20, 25 and 30, to check the homogeneity of the enrichment procedure during the experiments, with the variation observed below 2% in all cases (data not shown). Enriched *Artemia* metanauplii were collected in an appropriate size mesh screen, rinsed on a sieve (to remove residual emulsion) and blotted in filter paper before being frozen in liquid nitrogen and at -80 ºC

until analysis. The same treatment was used for 36-day old larvae at the end of the experiment. Eyes from 36 days old larvae from the different experimental treatments were dissected out and placed in chloroform / methanol (2:1, v/v) plus butylated hydroxytoluene (BHT) as antioxidant, for subsequent total lipid extraction and fatty acid composition analysis.

Dry weight, biometric values, survival, growth and biochemical composition determinations

Replicates of preweighed samples (approximately 500 mg wet weight) were maintained at 110 ºC for 24 h. The dry weights were determined after cooling in vacuo for 1 h. Biometric determinations were performed by micrometric analysis for light microscopy. Survival values presented are referred from hatching until the end of the experiment, since the delicacy of dentex larvae at the rotifer feeding stage made counting of larvae prior to the *Artemia* feeding stage impractical. Growth was assessed by measuring the Specific Growth Rate (SGR) as $\%\cdot day^{-1}$ (Wootton, 1990) and Thermal Growth Coefficient (TGC) (Cho, 1992). Protein content was determined by the Folin-phenol reagent method, according to Lowry et al., 1951. Total lipid contents were determined gravimetrically after extraction as described below. Carbohydrate contents were determined by a colorimetric method using the phenol-sulphuric acid reagent (Dubois et al., 1956). Ash contents were measured gravimetrically after total combustion in a furnace at 550 ºC. The gross energy content was calculated from the biochemical composition using values of 5.65, 9.45 and 4.20 kcal/g for protein, lipid and carbohydrates respectively (Henken et al., 1986).

Total lipid extraction, lipid class separation and quantification

Total lipid was extracted after homogenization in chloroform/methanol (2:1, v/v) containing 0.01% butylated hydroxytoluene (BHT) as antioxidant, basically according to Folch et al. (1957). Lipid classes were separated by high-performance thin-layer chromatography (HPTLC) on silica gel 60 plates, using the single-dimension double-

development method described previously (Tocher and Harvie, 1988; Olsen and Henderson, 1989). The classes were quantified by charring (Fewster et al., 1969) followed by calibrated densitometry using a Shimadzu CS-9001PC dual-wavelength flying spot scanner (Olsen and Henderson, 1989).

Total lipid and lipid classes fatty acid analyses

Lipid classes, phosphatidylcholine (PC), phosphatidylethanolamine (PE) and TAG were separated on 20 x 20 cm TLC plates using the above developing solvents. The separated classes were visualized by using 1% 2´,7´-dichlorofluoresceine in 98% methanol and viewing under UV light. Fatty acid methyl esters (FAME) from total lipids and lipid classes, scraped from the plates, were prepared by acid-catalyzed transmethylation for 16 h at 50ºC, using tricosanoic acid (23:0) as internal standard (Christie, 1989). FAME were extracted and purified as described previously (Tocher and Harvie,1988) and were separated in a Hewlett-Packard 5890A Series II gas chromatograph equipped with a chemically bonded (PEG) Supelcowax-10 fused-silica wall-coated capillary column (30 m x 0.32 mm i. d., Supelco Inc., Bellefonte, USA), "on column" injection system and flame ionization detection. Hydrogen was used as the carrier gas with an oven thermal gradient from an initial 50ºC to 180 ºC at 25ºC/min and then to a final temperature of 235 ºC at 3ºC/min. The final temperature was maintained for 10 min. Individual FAME were identified by comparison with known standards and quantified by means of a direct-linked PC and Hewlett-Packard ChemStation software.

Measurements of thiobarbituric acid reactive substances (TBARS)

The measurements of TBARS in triplicate samples were carried out using a method adapted from that used by Burk et al. (1980). Up to 20-30 mg of tissue per sample was homogenized in 1.5 ml of 20% trichloroacetic acid (w/v) containing 0.05 ml of 1% BHT in methanol. To this was added 2.95 ml of freshly prepared 50 mM thiobarbituric acid solution. The reagents were mixed in a stoppered test tube and heated at 100º C for 10 min. After cooling and removing protein precipitates by centrifugation at 2000 rpm, the

supernatant was read in a spectrophotometer at 532 nm. The absorbance was recorded against a blank at the same wavelength. The concentration of TBA-malondialdehyde (MDA) expressed as nmol MDA/g of tissue was calculated using the extinction coefficient 0.156 $\mu M^{-1}cm^{-1}$.

Determination of vitamin E content

Vitamin E concentrations (as tocopherol plus alpha-tocopheryl esters) were measured in tissue samples using high-performance liquid chromatography (HPLC). Samples were weighed, homogenized and saponified as described by Bieri (1969), but using a single-step hexane extraction (Bell et al, 1987). HPLC analysis was performed using a 250 x 2 mm reverse phase Spherisorb ODS2 column (Sigma Chemical Co, St. Louis MO, USA) essentially as described by Carpenter (1979). The mobile phase was 98% methanol pumped at 0.2 ml/min, the effluent from the column was monitored at a UV wavelength of 293 nm and quantitation achieved by comparison with (±)-alpha-tocopherol (Sigma Chemical CO, St. Louis, MO, USA) as external standard (10 μg/ml).

Determination of catalase, SOD, GPX, GST and GR activities in larval homogenates

Samples of larvae were homogenized in 9 volumes of 20mM Phosphate buffer pH 7.4, 1mM EDTA and 0.1% Triton X-100 and the homogenates centrifuged at 600 x g, to remove debris, and the resultant supernatants used directly for enzyme assays. Catalase (EC 1.11.1.6) activity was measured by following the reduction of hydrogen peroxide at 240 nm using the extinction coefficient 0.04 $mM^{-1}cm^{-1}$ (Beers and Sizer, 1952). Immediately before assay, 50 ml of 67 mM potassium phosphate buffer pH 7.0 was mixed with 80 μl of 30% (v/v) hydrogen peroxide. The assay cuvette (quartz) contained 3.0 ml of the above buffered hydrogen peroxide solution plus 25 μl of sample. Total SOD (EC 1.15.1.1) activity was assayed by measuring the inhibition of the oxygen-dependent oxidation of adrenalin (epinephrine) to adenochrome by xanthine oxidase plus xanthine (Panchenko et al., 1975). Plastic mini-cuvettes containing 0.5 ml of 100 mM potassium phosphate buffer pH 7.8 / 0.1 mM EDTA, 200 μl adrenaline, 200 μl xanthine

and 50 μl distilled water (uninhibited control) or 50 μl sample were prepared and the reaction initiated by the addition of 10 μl xanthine oxidase. The reaction was followed at 480 nm and 1 unit of superoxide dismutase activity is described as the amount of the enzyme which inhibited the rate of adenochrome production by 50%. GPX (EC 1.11.1.9) was assayed by following the rate of NADPH oxidation at 340 nm by the coupled reaction with glutathione reductase (Bell et al. 1985). Plastic mini-cuvettes containing 0.75 ml of 60 mM potassium phosphate buffer pH 7.4/1 mM EDTA/2 mM sodium azide, 50 μl reduced glutathione, 100 μl NADPH and 5 μl glutathione reductase were prepared. The basal reaction was initiated by the addition of either 50 μl hydrogen peroxide solution or 50 μl cumene hydroperoxide (as substrates for selenium-dependent and total GPX activities). The non-enzymic rate without sample added was measured for later subtraction. Sample (50 μl) was added and the assay continued by measuring absorbance at 340 nm with specific activities determined using the extinction coefficient of 6.22 $mM^{-1}cm^{-1}$. GST activity was determined by following the formation of glutathione-CDNB adduct at 340 nm. Standard plastic cuvettes containing 2.5 ml of 120 mM potassium phosphate buffer pH 6.5, 100 ml GSH and 100 ml CDNB were prepared and the reaction initiated by the addition of 50 μl sample. Specific activities were determined using an extinction coefficient of 9.6 $mM^{-1}cm^{-1}$ (Habig et al 1974). GR (EC 1.6.4.2) activity was assayed as described by Racker (1955) by measuring the oxidation of NADPH at 340 nm using the extinction coefficient 6.22 $mM^{-1}cm^{-1}$. Plastic mini-cuvettes containing 0.6 ml of 0.2M potassium phosphate buffer pH7.0/2 mM EDTA, 200 μl oxidised glutathione and 100 μl NADPH were prepared and the reaction initiated by the addition of 100 μl of sample. Protein content in the egg and larval homogenates was determined by the Folin-phenol reagent method, according to Lowry et al. (1951) following digestion in NaOH/SDS.

Materials

High-performance thin-layer chromatography (HPTLC) plates (10 x 10 x 0.15 mm), pre-coated with silica gel 60 were obtained from Merck (Darmstadt, Germany). All solvents were of HPLC grade and were obtained from Romil Technokroma (Barcelona, Spain).

EDTA, adrenaline, xanthine, potassium cyanide, reduced and oxidised glutathione, NADPH, hydrogen peroxide, cumene hydroperoxide, 1-chloro 2,4 dinitrobenzene, oxidized glutathione, sodium azide, glutathione reductase, xanthine oxidase and BHT were purchased from Sigma Chemical Co. Ltd., Alcobendas, Madrid, Spain.

Statistical analysis

Results are presented as means ± SD (n = 3 or 4). The data were checked for homogeneity of the variances by the Bartlett test and, where necessary, the data were arc-sin transformed before further statistical analysis. Differences between mean values were analyzed by one-way analysis of variance (ANOVA), followed when pertinent by a multiple comparison test (Tukey). Differences were reported as statistically significant when $P < 0.05$. Changes in the variables during the experimental period were studied by linear and non linear regression analysis (Zar, 1984).

Results

Fatty acid composition of Artemia diets

The (n-3) HUFA level in *Artemia* ranged from 0.72 to 6.23% of the diet dry weight, with values for EPA and DHA ranging from 0.55 to 4.16 % and from 0.01 to 1.54 % of the diet dry weight, respectively (Table 2). The enrichment markedly affected the fatty acid composition of *Artemia* and the (n-3) HUFA level in *Artemia* increased with increasing proportion of marine fish oil up to 60% (6.23% of the dry weight) in the emulsion mixture but it was not increased by using 100% fish oil emulsion. The total lipid contents of the *Artemia* diets ranged from 21.4 to 26.4 % on a dry weight basis but were not significantly different ($p < 0.05$). The (n-3)/(n-6) HUFA ratio ranged from 3.8 to 12.7 while the DHA/EPA ratio ranged from 0.06 to 0.37 (data not shown).

Gross composition, lipid class composition and fatty acid composition of dentex larvae

The gross composition and lipid class composition of dentex larvae fed the different dietary treatments are shown in Table 3.Total protein content presented values of about 60 % of the dry weight and did not show significant differences among treatments. Total lipid content showed a significantly lower value in larvae fed with treatment A. The nutritional status, indicated by the TAG/cholesterol (C) ratio, showed its highest value from treatment C onwards with *Artemia* containing an (n-3) HUFA level of 3.97 % on a dry weight basis. Larval fatty acid composition was considerably influenced by the enriched *Artemia* fatty acid content (Table 4). The (n-3) HUFA content in larvae from the different dietary treatments increased proportionally and was positively correlated to (n-3) HUFA content in the *Artemia* diets ($r^2 = 0.96$; $p < 0.01$). A similar trend was observed when comparing the (n-3) HUFA composition of eye total lipids and (n-3) HUFA content in *Artemia*, with maximal values for DHA and total (n-3) HUFA in eyes attained with *Artemia* dietary treatments D and E (Table 5).

Growth rate

Enrichment of *Artemia* with grading levels of (n-3) HUFA had a positive effect on growth of dentex larvae (Table 6). Maximal larval total length, individual larval dry weight, SGR and TGC were achieved with treatment C which contained 3.97 % (n-3) HUFA on a dry weight basis ($p<0.05$). Higher levels of (n-3) HUFA in *Artemia* (treatments D and E) presented similar or significantly lower values than with treatment C for the various growth variables. No significant differences were detected in survival among all treatments.

Dietary and larval vitamin E and malondialdehyde (MDA) levels and larval antioxidant enzyme activities

Vitamin E content in *Artemia* increased with increasing proportion of the ICES fish oil emulsion in the enrichment as the level of this vitamin is increased in diets with increased values of (n-3) HUFA (Table 6). MDA values in the *Artemia* diets were similar with the exception of treatment E which was 4-5 fold significantly (Table 6). Vitamin E

contents in dentex larvae were similar in all treatments with the exception of treatment E which showed significantly lower values. MDA levels in dentex larvae showed significantly higher values with Artemia in treatments D and E.

Antioxidant enzyme activities in dentex larvae

The activities of catalase, SOD and Se-dependent GPX did not show significant differences among the different dietary treatments. Se-independent GPX represented between 2.8 and 13.8 % of total GPX activities but no correlation was observed between experimental treatments and any other variable measured. GST showed significantly higher values with treatment D and GR showed only very low levels of activity for all treatments.

Discussion

The common dentex, *D. dentex*, is a fast growing sparid fish which is also extremely sensitive to handling and/or stressful operations. This the primary reason for the limited survival data in the present study as we only measured overall survival from hatched egg to the end of the experimental period. The delicacy of the larvae made it impractical to determine larval numbers immediately prior to feeding enriched *Artemia*. Optimal growth of dentex larvae in the present study, as indicated by larval total length, individual dry weight, SGR and TGC, was achieved when dietary (n-3) HUFA) was 3.97% on a dry weight basis. In a similar study with another sparid, the red sea bream (*Pagrus major*), a requirement for (n-3) HUFA of at least 3.0% on a dry weight basis at the *Artemia* feeding stage was suggested (Izquierdo et al 1989). In a flatfish, the flounder *Paralichthys olivaceus*, the requirement for (n-3) HUFA at the *Artemia* feeding stage was estimated to be 3.5% on a dry weight basis (Izquierdo et al 1992). However, the requirement for (n-3) HUFA of the yellowtail *Seriola quinqueradiata*, a carangid pelagic fast-swiming marine fish, at the Artemia feeding stage was shown to be 3.9% on a dry weight basis (Furuita et al 1996), a value very similar to that determined in the present study for the common dentex larvae. However, the ratios of DHA/EPA in that study and

the present study were very similar (about 0.6), and may be suboptimal. Indeed, it has been suggested that marine fish larval feeds with ratios of DHA/EPA less than or equal to 1 are sub optimal, either by not providing sufficient DHA or by providing an undesirable excess of EPA (Sargent et al 1997). In consequence, it is reasonable to think that an oil source for *Artemia* enrichment with an DHA/EPA ratio above 1 could provide better results by decreasing the total (n-3) HUFA requirement.

It is also noteworthy that the TAG/C ratio, a condition index of the nutritional status for fish larvae (Fraser 1989), reached its maximum value when the dietary content of (n-3) HUFA was 3.97% on a dry weight basis. In contrast, maximal values of (n-3) HUFA in total lipids of dentex larvae and eyes were achieved at values of dietary (n-3) HUFA greater than the requirement estimated by performance and condition indicators. This may indicate a higher requirement for dietary DHA, which is critical for larval growth and neural and retinal development (Sargent et al 1993; 1995; Mourente 1996; Bell 1998), than that obtained in treatment C (0.88%). In recent studies with larval marine fish where DHA requirement has been determined the values obtained were above that estimated in the present study: between 0.95 and 1.62% for larval red sea bream (*P. major*) and between 1.39 and 2.63% for larval yellowtail (*S. quinqueradiata*), both at the *Artemia* feeding stage (Furuita et al 1996 a,b). In the present study, we obtained values for dietary DHA of 1.54% and 1.28% on a dry weight basis in treatments D and E but the performance of the fish was lower compared to that with treatment C. This could be due to an excess of dietary EPA over DHA, and may be related to EPAs ability to competitively inhibit the production of eicosanoids derived from arachidonic acid (AA; 20:4(n-6)) (Sargent et al 1995; Bell 1998).

The primary effect of vitamin E deficiency is increased lipid oxidation. In the present study, poorer performance of larvae was associated with increased dietary and larval MDA and decreased larval vitamin E. This indicated increased oxidation of (n-3) HUFA in *Artemia* and larval utilization of vitamin E with increasing levels of dietary (n-3) HUFA, particularly at supraoptimal levels of enrichment. This is a known risk when using Artemia enrichment procedures based on fish oil emulsions with high levels of (n-3) HUFA which are prone to autooxidation, especially under the conditions of vigorous aeration employed for prolonged periods (McEvoy et al 1995; Sargent et al 1997).

Therefore, the higher the (n-3) HUFA requirement of marine fish larvae, the higher will be the requirement for natural antioxidants, particularly alpha-tocopherol. Furthermore, as alpha-tocopherol in the enrichment medium is consumed in protecting (n-3) HUFA against peroxidation during the enrichment itself, the higher will be the requirement for alpha-tocopherol to be present during the enrichment procedure (Sargent et al 1997). The minimun requirement of vitamin E should be defined as the minimum dose necessary to maintain the body content of alpha-tocopherol at a level where growth and/or survival rates are not reduced (Hamre and Lie 1995a). In any case, the ratio of alpha-tocopherol to polyunsaturated fatty acid (PUFA) in the fish tissues is critical in protection against lipid oxidation, and may modulate the vitamin E requierement (Hamre and Lie 1995b). Uncontrolled lipid oxidation is normally low in vivo, but the rate may increase if the fish is subjected to oxidative stress. Defence against in vivo oxidation also includes a suite of antioxidant enzyme systems (catalase, SOD, total GPX, Se-GPX, GST and GR), endogenously synthetised antioxidants (glutathione, ubiquinone), and antioxidant nutrients (vitamins E, C and carotenoids). This study has demonstrated that dentex larvae at the *Artemia* feeding stage can metabolize hydrogen peroxide (catalase and Se-GPX) and O2-. (SOD), detoxify organic hydroxyperoxides (total GPX and Se-GPX), detoxify xenobiotic compounds containing electrophilic centers (GST) and convert oxidized glutathione to its reduced form (GR). However, the activities of these antioxidant enzymes in dentex larvae were generally not greatly affected by the dietary treatments in the present study. This suggests that the control of expression of all these antioxidant enzymes or their activity was not directly affected by the level of dietary (n-3) HUFA. It is possible that the activities of the enzymes measured in the present study were sufficient to deal with the level of oxidation stress experienced by the larvae and that none of the diets imposed a sufficiently great oxidation stress on the larvae although the decreased vitamin E and increased MDA levels suggest otherwise.

In conclusion, the present study investigated the requirements of *D. dentex* larvae for (n-3) HUFA at the *Artemia* feeding stage. Optimal growth and performance of the larvae was achieved when dietary (n-3) HUFA was 3.97% on a dry weight basis. Larvae fed *Artemia* enriched with apparently supra-optimal levels of (n-3) HUFA (5.67-6.23 %) showed significantly lower vitamin E contents and higher MDA levels indicating

increased oxidative stress and poorer performance. However, despite indications of increased oxidative stress with higher levels of dietary (n-3) HUFA, the activities of antioxidant enzymes in the larvae were generally not greatly affected. The study underlines the need for a balance between growth-promoting essential fatty acid (EFA) qualities of (n-3) HUFA and their potentially growth-inhibiting (prooxidant) qualities which must be counter-balanced with adequate dietary antioxidants.

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Table 1
Total lipid content (dry wt.%) and total lipid fatty acid content (μg fatty acid (mg dry weight)$^{-1}$) of the emulsions and emulsion mixtures used as enrichers for *Artemia*. Results are means ± SD ($n = 3$). Values within a row with different superscript letters were significantly different ($P < 0.05$). See text for explanation of the different dietary treatments. HUFA = highly unsaturated fatty acids ≥ 20:3. nd = Not detected. Totals include some minor components not shown

Fatty acid	Treatment				
	A	B	C	D	E
12:0	369.9 ± 8.7^{a}	247.4 ± 9.6^{b}	228.9 ± 6.8^{b}	115.7 ± 3.2^{c}	10.7 ± 1.4^{d}
14:0	186.5 ± 6.9^{a}	137.0 ± 7.2^{b}	113.3 ± 5.8^{c}	58.4 ± 2.6^{d}	14.3 ± 0.4^{e}
15:0	16.4 ± 1.6^{a}	11.7 ± 1.8^{ab}	13.9 ± 1.2^{a}	8.3 ± 0.6^{b}	17.5 ± 1.3^{a}
16:0	111.8 ± 6.6^{a}	88.1 ± 3.9^{ab}	70.6 ± 5.3^{b}	42.9 ± 1.9^{c}	19.8 ± 0.8^{d}
17:0	1.8 ± 0.3	1.7 ± 0.2	1.8 ± 0.2	2.1 ± 0.3	2.1 ± 0.3
18:0	35.1 ± 1.2^{a}	28.7 ± 1.3^{ab}	26.1 ± 1.1^{b}	23.7 ± 0.9^{bc}	21.7 ± 0.9^{c}
20:0	1.6 ± 0.1^{a}	1.4 ± 0.1^{ab}	1.9 ± 0.1^{a}	3.2 ± 0.1^{c}	4.4 ± 0.2^{d}
22:0	nd	nd	1.3 ± 0.1^{a}	1.9 ± 0.2^{b}	2.6 ± 0.3^{c}
Total saturated	723.1 ± 7.8^{a}	514.9 ± 6.4^{b}	458.0 ± 6.3^{c}	257.6 ± 2.3^{d}	93.5 ± 1.1^{e}
16:1$n-9$	2.3 ± 0.2^{a}	1.6 ± 0.1^{b}	2.4 ± 0.2^{a}	2.7 ± 0.3^{ac}	3.1 ± 0.2^{c}
16:1$n-7$	2.0 ± 0.1^{a}	2.1 ± 0.1^{a}	2.4 ± 0.2^{a}	3.6 ± 0.2^{b}	4.6 ± 0.3^{c}
18:1$n-9$	81.4 ± 2.3^{a}	67.9 ± 2.5^{b}	60.2 ± 2.6^{b}	58.8 ± 2.5^{b}	57.6 ± 1.7^{b}
18:1$n-7$	2.6 ± 0.3^{a}	2.2 ± 0.2^{a}	4.8 ± 0.3^{b}	9.6 ± 0.5^{c}	13.3 ± 0.6^{d}
20:1$n-9$	1.7 ± 0.1^{a}	2.5 ± 0.2^{b}	6.5 ± 0.3^{c}	21.3 ± 1.2^{d}	33.1 ± 1.4^{e}
22:1	nd	2.4 ± 0.1^{a}	5.5 ± 0.2^{b}	17.4 ± 0.6^{c}	26.5 ± 0.8^{d}
Total monoenes	90.6 ± 1.9^{a}	79.4 ± 0.9^{b}	83.0 ± 1.2^{b}	120.8 ± 2.8^{c}	149.9 ± 2.3^{d}
18:2$n-6$	52.2 ± 1.8^{a}	42.2 ± 2.3^{b}	36.8 ± 1.2^{bc}	33.3 ± 1.2^{c}	29.5 ± 0.9^{cd}
18:3$n-3$	4.3 ± 0.3^{a}	4.2 ± 0.2^{a}	4.0 ± 0.3^{a}	4.8 ± 0.3^{b}	5.8 ± 0.3^{c}
18:4$n-3$	nd	nd	1.2 ± 0.1^{a}	5.0 ± 0.9^{b}	7.0 ± 0.7^{c}
20:2$n-6$	nd	nd	nd	1.6 ± 0.1^{a}	2.5 ± 0.1^{b}
20:3$n-6$	nd	nd	nd	1.2 ± 0.1	1.3 ± 0.1
20:4$n-6$	nd	0.1 ± 0.1^{a}	2.8 ± 0.2^{b}	7.7 ± 0.3^{c}	11.9 ± 0.4^{d}
20:3$n-3$	nd	nd	nd	1.4 ± 0.2^{a}	2.1 ± 0.1^{b}
20:4$n-3$	nd	nd	2.2 ± 0.3^{a}	6.8 ± 0.2^{b}	10.3 ± 0.3^{c}
20:5$n-3$	nd	13.1 ± 0.3^{a}	49.6 ± 2.4^{b}	148.0 ± 4.2^{c}	230.7 ± 6.8^{d}
22:5$n-6$	nd	nd	1.9 ± 0.3^{a}	4.7 ± 0.3^{b}	7.5 ± 0.3^{c}
22:5$n-3$	nd	3.8 ± 0.2^{a}	9.4 ± 0.4^{b}	30.4 ± 0.9^{c}	48.1 ± 1.4^{d}
22:6$n-3$	nd	11.3 ± 0.3^{a}	40.1 ± 1.8^{b}	125.3 ± 4.3^{c}	196.4 ± 6.9^{d}
Total polyenes	64.6 ± 2.1^{a}	87.5 ± 3.7^{b}	157.5 ± 6.4^{c}	388.4 ± 6.9^{d}	579.2 ± 5.8^{e}
Total $n-6$	44.8 ± 2.1^{a}	48.6 ± 1.9^{a}	46.5 ± 2.3^{a}	60.5 ± 2.4^{b}	70.6 ± 1.8^{c}
Total $n-3$	9.8 ± 0.2^{a}	38.9 ± 1.6^{b}	110.9 ± 3.7^{c}	327.9 ± 5.9^{d}	508.6 ± 4.7^{e}
HUFA $n-6$	nd	2.8 ± 0.2^{a}	7.3 ± 0.3^{b}	23.6 ± 0.4^{c}	35.9 ± 0.6^{d}
HUFA $n-3$	nd	28.3 ± 1.1^{a}	101.3 ± 2.2^{b}	313.8 ± 3.2^{c}	490.3 ± 3.8^{d}
Total lipid (%)	62.1 ± 3.1	64.5 ± 2.2	63.5 ± 1.7	64.1 ± 1.1	63.8 ± 2.2

Table 2
Total lipid content (dry wt.%) and total lipid fatty acid content (μg fatty acid (mg dry weight)$^{-1}$) of *Artemia* metanauplii enriched with different emulsion treatment. Results are means ± SD ($n = 12$). Values within a row with different superscript letters were significantly different ($P < 0.05$). See text for explanation of the different dietary treatments. HUFA = highly unsaturated fatty acids ≥ 20:3. Totals include some minor components not shown

Fatty acid	Treatment				
	A	B	C	D	E
12:0	13.4 ± 1.8^{a}	13.2 ± 3.6^{a}	10.6 ± 4.3^{a}	3.5 ± 1.3^{b}	0.1 ± 0.1^{c}
14:0	12.2 ± 0.9^{a}	11.8 ± 2.4^{a}	11.6 ± 2.0^{a}	4.9 ± 0.7^{b}	0.8 ± 0.1^{c}
15:0	2.1 ± 0.2^{a}	2.6 ± 0.3^{b}	3.2 ± 0.3^{c}	2.4 ± 0.4^{ab}	2.6 ± 0.4^{b}
16:0	20.0 ± 0.9^{a}	19.4 ± 1.9^{a}	19.7 ± 2.0^{a}	16.0 ± 1.0^{b}	13.0 ± 0.6^{c}
17:0	1.7 ± 0.1^{ab}	1.5 ± 0.1^{bc}	1.5 ± 0.1^{c}	1.6 ± 0.1^{ab}	1.7 ± 0.1^{a}
18:0	8.7 ± 0.5^{a}	8.2 ± 1.0^{ab}	8.1 ± 0.9^{ab}	7.6 ± 0.6^{b}	8.4 ± 0.6^{ab}
20:0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
22:0	0.4 ± 0.2^{a}	0.5 ± 0.1^{ab}	0.7 ± 0.3^{b}	0.5 ± 0.1^{ab}	0.6 ± 0.0^{ab}
Total saturated	58.7 ± 4.1^{a}	57.5 ± 8.5^{a}	55.6 ± 9.0^{a}	36.9 ± 3.3^{b}	27.5 ± 1.5^{c}
16:1$n-9$	0.9 ± 0.1^{a}	0.8 ± 0.1^{ab}	0.8 ± 0.1^{ab}	0.7 ± 0.0^{b}	0.7 ± 0.0^{b}
16:1$n-7$	4.0 ± 0.4	3.9 ± 0.5	4.1 ± 0.4	4.2 ± 0.3	4.0 ± 0.3
18:1$n-9$	34.1 ± 1.9^{a}	34.1 ± 2.0^{a}	36.2 ± 1.5^{a}	34.5 ± 2.3^{a}	30.4 ± 1.2^{b}
18:1$n-7$	10.9 ± 0.8^{a}	10.6 ± 0.8^{a}	10.8 ± 0.4^{a}	11.8 ± 0.6^{b}	11.9 ± 0.6^{b}
20:1$n-9$	0.8 ± 0.1^{a}	0.8 ± 0.1^{a}	1.1 ± 0.1^{b}	1.8 ± 0.1^{c}	2.0 ± 0.1^{d}
22:1	0.4 ± 0.3^{ab}	0.2 ± 0.1^{a}	0.4 ± 0.1^{b}	0.4 ± 0.1^{ab}	0.5 ± 0.1
Total monoenes	51.0 ± 3.3^{abc}	50.3 ± 3.3^{ab}	53.7 ± 2.1^{bc}	53.9 ± 2.7^{a}	50.2 ± 1.8^{c}
18:2$n-6$	15.3 ± 1.3^{a}	14.8 ± 0.9^{a}	15.7 ± 0.5^{a}	13.1 ± 1.0^{b}	9.1 ± 0.3^{c}
18:3$n-3$	27.0 ± 1.8	25.0 ± 1.8	25.3 ± 2.3	25.2 ± 2.3	24.5 ± 2.3
18:4$n-3$	3.2 ± 0.4^{ab}	2.9 ± 0.2^{a}	3.6 ± 0.3^{b}	4.1 ± 0.3^{c}	3.4 ± 0.3^{b}
20:2$n-6$	0.1 ± 0.1^{a}	0.1 ± 0.1^{a}	0.4 ± 0.0^{b}	0.5 ± 0.0^{b}	0.6 ± 0.1^{b}
20:3$n-6$	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.1	0.3 ± 0.0	0.3 ± 0.0
20:4$n-6$	1.4 ± 0.1^{a}	1.7 ± 0.2^{b}	2.5 ± 0.2^{c}	3.4 ± 0.2^{d}	3.4 ± 0.1^{d}
20:3$n-3$	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	0.7 ± 0.1
20:4$n-3$	0.6 ± 0.1^{a}	0.7 ± 0.0^{b}	1.2 ± 0.0^{c}	1.6 ± 0.1^{d}	1.5 ± 0.0^{e}
20:5$n-3$	5.5 ± 1.0^{a}	12.9 ± 2.6^{b}	27.4 ± 3.9^{c}	41.6 ± 3.2^{d}	39.0 ± 1.5^{d}
22:5$n-6$	0.2 ± 0.1^{a}	0.4 ± 0.1^{b}	0.4 ± 0.1^{b}	0.4 ± 0.0^{b}	0.4 ± 0.0^{b}
22:5$n-3$	0.3 ± 0.1^{a}	0.6 ± 0.2^{b}	1.7 ± 0.2^{c}	2.9 ± 0.2^{d}	2.6 ± 0.1^{d}
22:6$n-3$	0.1 ± 0.1^{a}	3.1 ± 0.9^{b}	8.8 ± 1.2^{c}	15.4 ± 1.1^{d}	12.8 ± 0.5^{e}
Total polyenes	58.3 ± 3.1^{a}	67.0 ± 4.8^{b}	91.9 ± 5.7^{c}	114.2 ± 8.2^{d}	103.1 ± 4.0^{e}
Total $n-6$	18.8 ± 1.5^{a}	19.0 ± 0.9^{ab}	21.3 ± 0.8^{c}	20.3 ± 1.4^{bc}	16.0 ± 0.4^{d}
Total $n-3$	39.5 ± 2.1^{a}	48.0 ± 4.2^{b}	70.6 ± 5.0^{c}	93.9 ± 7.0^{d}	87.0 ± 3.7^{e}
HUFA $n-6$	1.9 ± 0.1^{a}	2.5 ± 0.3^{b}	3.5 ± 0.3^{c}	4.9 ± 0.3^{d}	4.7 ± 0.1^{d}
HUFA $n-3$	7.2 ± 0.9^{a}	18.0 ± 3.5^{b}	39.7 ± 5.3^{c}	62.3 ± 4.6^{d}	56.7 ± 2.1^{d}
Total lipid (%)	21.4 ± 2.1	23.3 ± 2.1	26.4 ± 2.7	26.2 ± 1.4	23.7 ± 1.8

Table 3
Gross composition (dry wt.%) and lipid class composition (total lipid percentage) of *D. dentex* larvae fed different experimental dietary treatments. Results are means ± SD ($n = 3$). Values within a row with different superscript letters were significantly different ($P < 0.05$). TAG = Triacylglycerol; C = Cholesterol

	Treatment				
	A	B	C	D	E
Protein	61.5 ± 1.5	62.5 ± 2.1	63.6 ± 1.3	62.2 ± 2.3	61.5 ± 1.6
Lipid	16.3 ± 0.4^{a}	18.2 ± 0.1^{b}	18.1 ± 0.4^{b}	18.1 ± 0.1^{b}	17.8 ± 0.4^{b}
Carbohydrate	2.6 ± 0.3^{a}	2.8 ± 0.4^{a}	4.2 ± 0.3^{b}	2.7 ± 0.1^{a}	3.9 ± 0.2^{b}
Ash	19.5 ± 0.8^{a}	16.5 ± 1.1^{b}	14.1 ± 1.2^{c}	17.0 ± 0.9^{b}	16.8 ± 1.1^{b}
Sphingomyelin	2.4 ± 0.1^{a}	2.1 ± 0.4^{a}	1.3 ± 0.3^{b}	1.7 ± 0.1^{ab}	2.0 ± 0.0^{ab}
Phosphatidylcholine	19.6 ± 1.0^{ab}	21.1 ± 2.7^{a}	15.8 ± 1.4^{b}	19.1 ± 0.7^{ab}	18.6 ± 1.1^{ab}
Phosphatidylserine	5.8 ± 0.2^{a}	4.9 ± 0.5^{ab}	2.6 ± 0.4^{c}	3.6 ± 0.3^{b}	3.7 ± 0.1^{b}
Phosphatidylinositol	4.4 ± 0.1^{a}	4.2 ± 0.4^{a}	2.6 ± 0.2^{b}	3.2 ± 0.2^{b}	3.2 ± 0.3^{b}
Phosphatidic acid/cardiolipin	3.1 ± 0.1^{a}	2.8 ± 0.2^{a}	1.6 ± 0.3^{b}	2.3 ± 0.4^{ab}	2.5 ± 0.1^{a}
Phosphatidylethanolamine	15.9 ± 0.1^{a}	15.4 ± 1.5^{a}	11.1 ± 0.8^{b}	12.4 ± 0.9^{b}	12.3 ± 0.2^{b}
Lyso-phosphatidylcholine	0.3 ± 0.2	0.5 ± 0.2	0.1 ± 0.1	0.1 ± 0.2	0.1 ± 0.1
Pigments	3.6 ± 0.3^{a}	2.6 ± 0.4^{ab}	2.3 ± 0.3^{b}	2.7 ± 0.3^{ab}	1.9 ± 0.2^{b}
Cholesterol	23.2 ± 0.2^{a}	17.0 ± 0.4^{b}	17.2 ± 3.1^{b}	15.3 ± 0.1^{b}	14.9 ± 0.4^{b}
Free fatty acid	7.3 ± 0.4^{a}	4.1 ± 0.6^{b}	5.8 ± 0.8^{ab}	3.8 ± 0.6^{b}	4.3 ± 0.6^{b}
Triacylglycerol	8.9 ± 0.5^{a}	19.4 ± 4.5^{b}	35.8 ± 1.4^{c}	32.8 ± 2.2^{c}	32.7 ± 0.8^{c}
Sterol ester/wax ester	5.3 ± 0.8^{a}	5.8 ± 0.9^{a}	3.9 ± 0.7^{ab}	3.0 ± 0.3^{b}	3.8 ± 0.3^{ab}
Total polar lipid	55.2 ± 1.9^{a}	53.7 ± 5.6^{a}	37.4 ± 3.2^{b}	45.2 ± 2.8^{ab}	44.2 ± 0.9^{ab}
Total neutral lipid	44.8 ± 1.9^{a}	46.3 ± 5.6^{a}	62.6 ± 3.2^{b}	54.8 ± 2.8^{ab}	55.8 ± 0.9^{ab}
TAG/C	0.4 ± 0.1^{a}	1.1 ± 0.1^{b}	2.1 ± 0.1^{c}	2.1 ± 0.1^{c}	2.2 ± 0.1^{c}

Table 4
Total lipid fatty acid composition (wt.%) of *D. dentex* larvae fed with different dietary treatment. Results are means ± SD ($n = 3$). Values within a row with different superscript letters were significantly different ($P < 0.05$). See text for explanation of the different dietary treatments. HUFA = highly unsaturated fatty acids ≥ 20:3. Totals include some minor components not shown

Fatty acid	Treatment				
	A	B	C	D	E
12:0	0.4 ± 0.0^{ab}	0.9 ± 0.0^{b}	1.4 ± 0.7^{b}	0.6 ± 0.0^{ab}	0.1 ± 0.0^{a}
14:0	1.7 ± 0.0^{a}	2.9 ± 0.0^{b}	3.8 ± 0.1^{c}	1.7 ± 0.0^{a}	0.6 ± 0.1^{d}
15:0	14.6 ± 0.2^{a}	6.5 ± 0.3^{b}	5.5 ± 0.1^{c}	7.9 ± 0.2^{d}	6.2 ± 0.2^{b}
16:0	11.6 ± 0.1^{a}	12.9 ± 0.1^{b}	11.8 ± 0.1^{a}	10.8 ± 0.0^{c}	10.2 ± 0.2^{d}
17:0	0.0 ± 0.0	0.6 ± 0.1	0.6 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
18:0	6.2 ± 0.1^{a}	6.8 ± 0.0^{b}	5.7 ± 0.0^{b}	5.6 ± 0.0^{b}	6.2 ± 0.1^{a}
Total saturated	34.5 ± 0.3^{a}	30.6 ± 0.3^{b}	28.8 ± 0.9^{c}	26.5 ± 0.2^{d}	23.3 ± 0.4^{e}
16:1*n* − 7	2.0 ± 0.0^{a}	2.4 ± 0.1^{c}	2.3 ± 0.1^{c}	2.0 ± 0.0^{ab}	2.3 ± 0.1^{bc}
18:1*n* − 9	9.9 ± 0.1^{a}	14.0 ± 0.2^{b}	13.8 ± 0.0^{b}	11.4 ± 0.1^{b}	12.0 ± 0.2^{c}
18:1*n* − 7	4.3 ± 0.1^{a}	5.8 ± 0.1^{b}	5.3 ± 0.0^{c}	5.0 ± 0.1^{b}	5.9 ± 0.1^{b}
20:1*n* − 9	0.6 ± 0.0^{a}	0.8 ± 0.0^{b}	0.7 ± 0.0^{abc}	0.6 ± 0.0^{ab}	0.8 ± 0.1^{bc}
Total monoenes	16.7 ± 0.2^{a}	23.1 ± 0.1^{b}	22.2 ± 0.1^{c}	19.2 ± 0.1^{d}	21.0 ± 0.4^{e}
18:2*n* − 6	6.0 ± 0.1^{a}	6.7 ± 0.0^{b}	5.9 ± 0.0^{a}	4.1 ± 0.1^{c}	3.6 ± 0.1^{d}
18:3*n* − 3	5.2 ± 0.1^{a}	9.2 ± 0.1^{b}	8.2 ± 0.0^{c}	5.9 ± 0.0^{b}	7.5 ± 0.1^{c}
18:4*n* − 3	0.4 ± 0.0^{a}	0.9 ± 0.0^{b}	0.9 ± 0.0^{b}	0.8 ± 0.0^{b}	0.9 ± 0.0^{b}
20:2*n* − 6	0.7 ± 0.1^{a}	0.5 ± 0.1^{ab}	0.4 ± 0.1^{b}	0.4 ± 0.0^{ab}	0.4 ± 0.0^{ab}
20:4*n* − 6	2.5 ± 0.0^{a}	2.1 ± 0.0^{bc}	1.9 ± 0.0^{c}	2.3 ± 0.1^{ab}	2.3 ± 0.0^{ab}
20:3*n* − 3	0.7 ± 0.0^{a}	0.6 ± 0.0^{b}	0.5 ± 0.0^{c}	0.4 ± 0.0^{d}	0.4 ± 0.0^{e}
20:4*n* − 3	0.7 ± 0.0^{a}	0.7 ± 0.0^{ab}	0.5 ± 0.0^{d}	0.6 ± 0.0^{cd}	0.6 ± 0.0^{bc}
20:5*n* − 3	6.1 ± 0.1^{a}	8.8 ± 0.0^{b}	11.2 ± 0.0^{c}	12.9 ± 0.0^{d}	13.9 ± 0.1^{e}
22:5*n* − 6	1.2 ± 0.1^{a}	0.3 ± 0.0^{b}	0.0 ± 0.0	0.8 ± 0.0^{c}	0.6 ± 0.0^{d}
22:5*n* − 3	0.5 ± 0.0^{a}	0.8 ± 0.0^{b}	1.3 ± 0.0^{c}	1.9 ± 0.0^{d}	1.9 ± 0.0^{d}
22:6*n* − 3	1.2 ± 0.1^{a}	2.6 ± 0.1^{b}	4.5 ± 0.0^{c}	8.8 ± 0.0^{d}	8.7 ± 0.3^{d}
Total polyenes	33.5 ± 0.3^{a}	36.7 ± 0.2^{b}	39.2 ± 0.2^{c}	43.8 ± 0.1^{d}	45.1 ± 0.0^{e}
Unknown	15.2 ± 0.6^{a}	9.6 ± 0.3^{b}	9.8 ± 0.8^{b}	10.5 ± 0.2^{b}	10.5 ± 0.8^{b}
Total *n* − 6	13.6 ± 0.1^{a}	10.8 ± 0.1^{b}	9.6 ± 0.2^{c}	9.2 ± 0.2^{cd}	8.6 ± 0.1^{d}
Total *n* − 3	20.0 ± 0.2^{a}	25.8 ± 0.1^{b}	29.6 ± 0.4^{c}	34.6 ± 0.3^{d}	36.5 ± 0.1^{e}
HUFA *n* − 6	3.7 ± 0.0^{a}	2.4 ± 0.0^{b}	1.9 ± 0.0^{c}	3.1 ± 0.2^{b}	2.9 ± 0.0^{b}
HUFA *n* − 3	10.1 ± 0.1^{a}	13.6 ± 0.0^{b}	18.4 ± 0.3^{c}	25.0 ± 0.1^{d}	25.9 ± 0.1^{e}

Table 5
Total lipid fatty acid composition (wt.%) of the eyes of *D. dentex* larvae fed with different dietary treatment. Results are means ± SD ($n = 3$). Values within a row with different superscript letters were significantly different ($P < 0.05$). See text for explanation of the different dietary treatments. DMA, dimethyl acetal. HUFA = highly unsaturated fatty acids ≥ 20:3. Totals include some minor components not shown

Fatty acid	Treatment				
	A	B	C	D	E
12:0	0.1 ± 0.0^{a}	0.3 ± 0.0^{b}	0.4 ± 0.0^{c}	0.1 ± 0.0^{d}	0.0 ± 0.0^{a}
14:0	1.4 ± 0.0^{a}	1.9 ± 0.0^{b}	2.2 ± 0.0^{c}	1.1 ± 0.0^{d}	0.4 ± 0.0^{e}
15:0	1.7 ± 0.1^{a}	1.9 ± 0.1^{b}	1.3 ± 0.0^{c}	2.0 ± 0.0^{b}	1.2 ± 0.0^{c}
16:0	15.7 ± 0.1^{a}	14.1 ± 0.0^{b}	13.8 ± 0.0^{c}	12.8 ± 0.0^{d}	11.5 ± 0.0^{e}
17:0	0.4 ± 0.0^{a}	0.6 ± 0.0^{bc}	0.6 ± 0.0^{bc}	0.5 ± 0.0^{b}	0.7 ± 0.0^{c}
18:0	11.0 ± 0.1^{a}	9.7 ± 0.0^{b}	8.9 ± 0.0^{c}	8.5 ± 0.0^{d}	8.5 ± 0.0^{d}
20:0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
22:0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
Total saturated	30.7 ± 0.2^{a}	28.7 ± 0.1^{b}	27.7 ± 0.0^{c}	25.4 ± 0.0^{d}	22.7 ± 0.1^{e}
16:1*n* − 9	1.0 ± 0.0^{a}	0.7 ± 0.0^{b}	0.8 ± 0.0^{b}	0.6 ± 0.0^{c}	0.6 ± 0.0^{c}
16:1*n* − 7	1.5 ± 0.1^{ab}	1.6 ± 0.0^{ab}	1.7 ± 0.0^{a}	1.4 ± 0.1^{b}	1.5 ± 0.1^{ab}
18:1*n* − 9	12.2 ± 0.1^{a}	12.7 ± 0.1^{c}	14.0 ± 0.1^{d}	11.9 ± 0.0^{b}	12.0 ± 0.1^{ab}
18:1*n* − 7	4.7 ± 0.1^{a}	5.3 ± 0.0^{b}	5.6 ± 0.1^{c}	5.2 ± 0.0^{b}	5.9 ± 0.1^{d}
20:1*n* − 9	0.7 ± 0.0^{a}	0.6 ± 0.0^{ab}	0.6 ± 0.0^{b}	0.5 ± 0.0^{c}	0.7 ± 0.0^{ab}
20:1*n* − 7	0.2 ± 0.2	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
22:1	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
Total monoenes	20.5 ± 0.2^{a}	21.1 ± 0.1^{b}	23.0 ± 0.1^{c}	19.8 ± 0.1^{d}	21.0 ± 0.0^{b}
18:2*n* − 6	6.9 ± 0.1^{a}	5.7 ± 0.0^{b}	6.0 ± 0.0^{c}	3.6 ± 0.0^{d}	3.1 ± 0.1^{e}
18:3*n* − 3	4.4 ± 0.0^{a}	6.3 ± 0.0^{b}	7.1 ± 0.0^{c}	4.7 ± 0.1^{d}	6.0 ± 0.0^{e}
18:4*n* − 3	0.4 ± 0.1^{a}	0.6 ± 0.0^{b}	0.7 ± 0.0^{b}	0.7 ± 0.0^{b}	0.7 ± 0.0^{b}
20:2*n* − 6	0.4 ± 0.1^{a}	0.4 ± 0.0^{a}	0.4 ± 0.0^{a}	0.2 ± 0.0^{b}	0.2 ± 0.0^{b}
20:3*n* − 6	0.3 ± 0.0^{a}	0.1 ± 0.0^{b}	0.2 ± 0.0^{b}	0.1 ± 0.0^{c}	0.1 ± 0.0^{c}
20:4*n* − 6	3.6 ± 0.0^{a}	2.4 ± 0.0^{b}	2.3 ± 0.0^{c}	2.0 ± 0.0^{d}	2.1 ± 0.0^{e}
20:3*n* − 3	1.0 ± 0.0^{a}	1.0 ± 0.0^{b}	0.9 ± 0.0^{c}	0.5 ± 0.0^{d}	0.5 ± 0.0^{e}
20:4*n* − 3	1.1 ± 0.0^{a}	0.9 ± 0.0^{b}	0.8 ± 0.0^{c}	0.6 ± 0.0^{d}	0.7 ± 0.0^{e}
20:5*n* − 3	12.7 ± 0.1^{a}	13.5 ± 0.1^{c}	13.1 ± 0.1^{b}	12.8 ± 0.1^{ab}	13.6 ± 0.1^{c}
22:5*n* − 6	0.6 ± 0.0^{a}	0.4 ± 0.0^{b}	0.3 ± 0.0^{c}	0.2 ± 0.0^{cd}	0.2 ± 0.0^{d}
22:5*n* − 3	3.7 ± 0.0^{a}	3.9 ± 0.0^{b}	3.6 ± 0.0^{c}	4.0 ± 0.0^{d}	3.9 ± 0.0^{b}
22:6*n* − 3	5.4 ± 0.3^{a}	8.5 ± 0.0^{b}	7.3 ± 0.1^{c}	19.1 ± 0.2^{d}	17.6 ± 0.1^{e}
Total polyenes	43.5 ± 0.3^{a}	46.4 ± 0.2^{b}	45.4 ± 0.1^{c}	51.6 ± 0.3^{d}	52.1 ± 0.1^{d}
16:0DMA	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.1	0.3 ± 0.0
18:0DMA	0.9 ± 0.0^{a}	0.5 ± 0.0^{b}	0.5 ± 0.0^{b}	0.4 ± 0.0^{c}	0.4 ± 0.0^{c}
18:1DMA	0.6 ± 0.0^{a}	0.3 ± 0.0^{b}	0.3 ± 0.0^{b}	0.3 ± 0.0^{b}	0.4 ± 0.0^{b}
Total DMA	1.8 ± 0.0^{a}	1.1 ± 0.0^{bc}	1.0 ± 0.0^{b}	1.0 ± 0.0^{d}	1.1 ± 0.0^{c}
Unknown	3.5 ± 0.0	2.7 ± 0.3	2.9 ± 0.1	2.2 ± 0.3	3.1 ± 0.1
Total *n* − 6	13.5 ± 0.0^{a}	10.5 ± 0.1^{b}	10.6 ± 0.0^{b}	7.9 ± 0.1^{c}	7.6 ± 0.0^{d}
Total *n* − 3	30.0 ± 0.3^{a}	35.9 ± 0.2^{b}	34.7 ± 0.1^{c}	43.8 ± 0.2^{d}	44.6 ± 0.1^{e}
HUFA *n* − 6	5.3 ± 0.1^{a}	3.5 ± 0.0^{b}	3.3 ± 0.0^{c}	3.0 ± 0.0^{c}	3.2 ± 0.0^{d}
HUFA *n* − 3	23.9 ± 0.3^{a}	27.7 ± 0.1^{b}	25.6 ± 0.1^{c}	37.1 ± 0.3^{d}	36.3 ± 0.1^{e}

Table 6
Results of the dietary trial with *D. dentex* larvae at *Artemia* feeding stage enriched to give different levels of $n-3$ HUFA, showing dietary and larval oxidation status and larval antioxidant enzyme activities for the different experimental treatments. nd = Not detected. Mean values within horizontal rows bearing different superscript letters are significantly different ($P < 0.05$)

Treatment	A	B	C	D	E
Results of dietary trial					
Larval total length (mm)	10.2 ± 1.2^{a}	13.5 ± 1.8^{b}	16.4 ± 1.7^{c}	15.7 ± 1.2^{c}	13.7 ± 1.7^{b}
Dry wt.%	16.3 ± 0.4^{a}	18.2 ± 0.1^{b}	18.1 ± 0.4^{b}	18.1 ± 0.1^{b}	17.8 ± 0.4^{b}
Larval dry weight (mg)	2.4 ± 0.1^{a}	6.2 ± 2.8^{b}	10.9 ± 2.4^{c}	8.4 ± 0.6^{bc}	6.2 ± 1.7^{b}
Survival (%)	0.5 ± 0.4	1.0 ± 0.7	1.2 ± 0.1	1.5 ± 0.2	1.4 ± 0.7
SGR[1]	14.3 ± 0.2^{a}	18.3 ± 2.5^{b}	21.5 ± 1.1^{c}	20.3 ± 0.3^{bc}	18.7 ± 1.4^{b}
TGC[2]	20.7 ± 0.5^{a}	31.7 ± 7.7^{b}	42.1 ± 3.9^{c}	37.8 ± 1.3^{bc}	32.7 ± 4.2^{b}
Artemia diet					
Vitamin E[3]	401.4 ± 29.6^{a}	548.3 ± 15.6^{b}	598.5 ± 24.2^{b}	694.6 ± 24.7^{c}	1043.6 ± 37.5^{d}
MDA[4]	128.3 ± 19.1^{a}	125.0 ± 20.3^{a}	65.7 ± 9.7^{a}	117.5 ± 13.5^{a}	551.9 ± 61.0^{b}
D. dentex larvae					
Vitamin E[3]	108.8 ± 14.4^{a}	111.6 ± 16.9^{a}	114.2 ± 2.9^{a}	101.3 ± 5.1^{a}	71.6 ± 3.4^{b}
MDA[4]	80.9 ± 8.0^{a}	84.5 ± 6.1^{a}	106.2 ± 4.9^{a}	228.1 ± 13.3^{b}	209.6 ± 23.4^{b}
Antioxidant enzyme activities					
Catalase[5]	100.9 ± 1.8	73.7 ± 3.6	106.6 ± 28.5	97.1 ± 38.3	97.0 ± 16.1
Superoxide dismutase[6]	10.1 ± 3.1	8.2 ± 4.2	13.4 ± 0.4	9.6 ± 1.3	12.7 ± 1.2
Glutathione peroxidase[7]	87.5 ± 2.9	79.9 ± 0.6	79.5 ± 1.6	79.7 ± 4.9	93.1 ± 9.7
Se-dependent					
Total	98.6 ± 0.7^{a}	85.5 ± 1.3^{b}	92.3 ± 3.9^{ab}	88.7 ± 1.3^{ab}	95.8 ± 2.8^{ab}
GSH *S*-transferase[7]	63.5 ± 3.7^{a}	61.6 ± 3.3^{a}	60.9 ± 2.1^{a}	97.6 ± 3.1^{b}	67.6 ± 3.6^{a}
GSSG reductase[7]	9.6 ± 0.1	12.8 ± 4.3	13.4 ± 1.2	14.6 ± 2.3	8.6 ± 4.2

[1]SGR = specific growth rate (% day^{-1}); [2]TGC = thermal growth coefficient (ng dry weight °C^{-1} × day); [3]ng (mg dry weight)$^{-1}$; [4]nmol (g dry weight)$^{-1}$; [5]μmol min^{-1} mg^{-1} protein; [6]units mg^{-1} protein; [7]nmol min^{-1} mg^{-1} protein.