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Study of the $n-3$ highly unsaturated fatty acids 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 experimental emulsions containing (a) 500 mg g^{-1} $n-3$ HUFA, 0.6 DHA/EPA ratio, based on ethyl esters and (b) 0 mg g^{-1} $n-3$ HUFA, based on coconut oil, to give five dietary treatments which contained different levels of $n-3$ HUFA from 0.72 to 6.23 as dry wt.%. 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 content 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 enzyme in the larvae was generally not greatly affected by the dietary treatments in this study. A balance is required between growth-promoting

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essential fatty acids (EFA) qualities of $n-3$ HUFA and their potentially growth-inhibiting (pro-oxidant) qualities which must be counter-balanced with adequate dietary antioxidants. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: $n-3$ HUFA; Requirements; Antioxidant status; *Dentex dentex*; Fish larvae

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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 et 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 (Tulli and Tibaldi, 1997; Mourente et al., 1998). The significance of lipids at early stages of marine fish has been widely studied (Sargent et al., 1989, 1990, 1993; Watanabe and Kiron, 1994; Sargent, 1995; Mourente, 1996; Wiegand, 1996; Rainuzzo et al., 1997) and considerable advances have been made in understanding polyunsaturated fatty acid (PUFA) 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 (Furuita et al., 1996a,b; Izquierdo, 1996). To date, virtually all marine larviculture production systems rely on live foods, principally the rotifer *Brachionus plicatilis* and nauplii of *Artemia* which are deficient in $n-3$ 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 have dealt with the $n-3$ HUFA requirements of marine fish fed on live prey, in particular at the *Artemia* feeding stage (Izquierdo et al., 1989; Howell and Tzoumas, 1991; Izquierdo et al., 1992; Watanabe and Kiron, 1994; Furuita et al., 1996a,b; Izquierdo, 1996).

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 autoxidation (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 (TBARS)), 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

2.1. 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, Mallorca, Spain. Eggs were hatched in 400-l cylindro-conical tanks in an open circulation system (1–3 water exchange day^{-1}) at a density of 100 eggs l^{-1} at 19°C of temperature and 37‰ 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 Aquaculture, Belgium) at a density of 10–15 individuals ml^{-1} plus greenwater composed of *Nannochloropsis gaditana* and *Isochrysis galbana* (clone T-ISO) at a density of 80,000–100,000 cells ml^{-1}. From day 12 to day 15 *Artemia* nauplii grade AF480 (INVE Aquaculture) at 4–7 individuals ml^{-1} and from day 15 until day 36 (3 weeks) *Artemia* metanauplii grade EG (INVE Aquaculture), both enriched with the experimental emulsions, were fed.

2.2. Experimental emulsions and Artemia enrichment

The emulsions used to enrich *Artemia* were purchased from the Laboratory of Aquaculture and 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 ethyl esters and containing 500 mg $n-3$ HUFA (g dry weight)$^{-1}$ 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 just 100% coconut oil emulsion. Emulsion B was prepared by mixing 95% of coconut oil emulsion with 5% of the ethyl ester 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 Table 1.

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 25°C. The enrichment emulsions were prepared by mixing 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 aeration 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

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

(Merchie, 1996; Coutteau and Mourente, 1997). *Artemia* was given to fish twice daily, in the morning and in the afternoon.

2.3. Sample collection

Enriched *Artemia* metanauplii from the different experimental treatments were sampled at four 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°C until analysis. The same treatment was used for 15- (only for dry weight determination) and 36-day-old larvae at the end of the experiment. Whole larvae and eyes dissected out from 36-day-old larvae from the different experimental treatments were placed in chloroform/methanol (2:1, v/v) plus butylated hydroxytoluene (BHT) as antioxidant, for subsequent total lipid extraction and fatty acid composition analysis.

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

Replicates of preweighed samples (approximately 500 mg wet weight) were maintained at 110°C 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 % 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°C.

2.5. Total lipid extraction, lipid class separation and quantification

Total lipid was extracted after homogenization in chloroform/methanol (2:1, v/v) containing 0.01% 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).

2.6. Total lipid fatty acid analyses

Fatty acid methyl esters (FAME) from total lipids were prepared by acid-catalyzed transmethylation for 16 h at 50°C, 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 $\times$ 0.32 mm i.d., Supelco, 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 50°C to 180°C at 25°C min^{-1} and then to a final temperature of 235°C at 3°C min^{-1}. 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.

2.7. Measurements of 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 100°C for 10 min. After cooling, the tubes and removing protein precipitates by centrifugation at 2000 $\times$ g, the supernatant was read in an 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 per g of tissue was calculated using the extinction coefficient 0.156 μM^{-1} cm^{-1}.

2.8. Determination of vitamin E content

Vitamin E concentrations (as tocopherol plus α-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 $\times$ 2 mm reverse phase Spherisorb ODS2 column (Sigma, St. Louis, MO, USA) essentially as described by Carpenter (1979). The mobile phase was 98% methanol pumped at 0.2 ml min^{-1}, the effluent from the column was monitored at a UV wavelength of 293 nm and quantification achieved by comparison with (±)-α-tocopherol (Sigma) as external standard (10 μg ml^{-1}).

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

Samples of larvae were homogenised in 9 volumes of 20 mM phosphate buffer pH 7.4, 1 mM EDTA and 0.1% Triton X-100 and the homogenates centrifuged at 600 $\times$ 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 ml of 30% (v/v) hydrogen peroxide. The assay cuvette (quartz) contained 3.0 ml of 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 SOD activity is described as the amount of the enzyme which inhibits 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 GR (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 GR 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 μl GSH and 100 μl 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 0.2 M potassium phosphate buffer pH 7.0/2 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 larval homogenates was determined by the Folin-phenol reagent method, according to Lowry et al. (1951) following digestion in NaOH/SDS.

2.10. Statistical analysis

Results are presented as means $\pm$ 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 of the variables during the experimental period were studied by linear and non-linear regression analysis (Zar, 1984).

3. Results

3.1. 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

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

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 further increased by using 100% fish oil emulsion. The total lipid content 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.

3.2. Gross composition, lipid class composition and fatty acid composition of D. dentex larvae

The gross composition and the lipid class composition of *D. dentex* larvae fed the different experimental 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 just 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 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

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}

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).

3.3. 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*

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}

(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.

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] Se-dependent	87.5 ± 2.9	79.9 ± 0.6	79.5 ± 1.6	79.7 ± 4.9	93.1 ± 9.7
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.

3.4. Dietary and larval vitamin E and MDA levels and larval antioxidant enzyme activities

Vitamin E content in *Artemia* increased with the increase of the proportion of the ICES fish oil emulsion in the enrichment because the fish oil ethyl ester concentrate contained high vitamin E in comparison to the coconut oil (Dr. Peter Coutteau, personal communication) (Table 6). MDA values in *Artemia* diets were similar with the exception of treatment E which was 4–5 fold significantly higher (Table 6). Vitamin E content 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.

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 value with treatment D and GR showed only very low levels of activity for all treatments.

4. Discussion

The common dentex is a fast-growing sparid fish which is also extremely sensitive to handling and/or stressful operations. This is 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*. Larvae died gradually with the exception of two mortality peaks, the first one coincided with the 'point of no return' by inanition at days 8–9 after hatching when the larvae changed from lecitotrophia to exogenous feeding (rotifers) and the second at day 23 approx., when larvae changed from rotifer to *Artemia* nauplii diet, then larvae stabilized and started to eat enriched *Artemia* metanauplii.

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, a requirement for $n-3$ HUFA of at least 3.0% on a dry weight basis at *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, a carangid pelagic fast-swimming marine fish, at the *Artemia* feeding stage was shown to be 3.9% on a dry weight basis (Furuita et al., 1996b), 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 sub optimal. 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 the 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 and between 1.39 and 2.63% for larval yellowtail, both at the *Artemia* feeding

stage (Furuita et al., 1996a,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; $20{:}4n-6$) (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 autoxidation, 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 α-tocopherol. Furthermore, as α-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 α-tocopherol to be present during the enrichment procedure (Sargent et al., 1997). The minimum requirement of vitamin E should be defined as the minimum dose necessary to maintain the body content of α-tocopherol at a level where growth and/or survival are not reduced (Hamre and Lie, 1995a). In any case, the ratio of α-tocopherol to PUFA in the fish tissues is critical in protection against lipid oxidation, and may modulate the vitamin E requirement (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 enzymes (catalase, SOD, total GPX, Se-GPX, GST and GR), endogenously synthesised 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), O_2^- (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 suggest 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 was 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 (pro-oxidant) qualities which must be counter-balanced with adequate dietary antioxidants.

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References

Beers, R.F., Sizer, I.W., 1952. Spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J. Biol. Chem. 195, 133–140.

Bell, J.G., 1998. Current aspects of lipid nutrition in fish farming. In: Black, K.D., Pickering, A.D. (Eds.), Biology of Farmed Fish. Sheffield Academic Press, England, pp. 114–145.

Bell, J.G., Cowey, C.B., Adron, J.W., Shanks, A.M., 1985. Some effects of vitamin E and selenium deprivation on tissue enzyme levels and indices of tissue peroxidation in rainbow trout (*Salmo gairdneri*). Br. J. Nutr. 53, 149–157.

Bell, J.G., Cowey, C.B., Adron, J.W., Pirie, B.J.S., 1987. Some effects of selenium deficiency on enzyme activities and indices of tissue peroxidation in Atlantic salmon parr (*Salmo salar*). Aquaculture 65, 43–54.

Bieri, J.G., 1969. Chromatography of tocopherols. In: Marinetti, G.V. (Ed.), Lipid Chromatographic Analysis, Vol. 2. Marcel Dekker, New York, p. 459.

Burk, R.F., Trumble, M.J., Lawrence, R.A., 1980. Rat hepatic cytosolic GSH-dependent enzyme protection against lipid peroxidation in the NADPH microsomal lipid peroxidation system. Biochim. Biophys. Acta 618, 35–41.

Carpenter, A.P., 1979. Determination of tocopherols in vegetable oils. J. Am. Oil Chem. Soc. 56, 668–671.

Cataudella, S., Crosetti, D., Marino, G., 1995. The sea breams. In: Nash, C.E., Novotny, A.J. (Eds.), Production of Aquatic Animals: Fishes. Elsevier, Amsterdam, pp. 289–303.

Cho, C.Y., 1992. Feeding systems for rainbow trout and other salmonids with reference to current estimates of protein and energy requirements. Aquaculture 100, 107–123.

Christie, W.W., 1989. Gas Chromatography and Lipids: A Practical Guide, 1st edn. The Oily Press, Ayr, Scotland.

Coutteau, P., Mourente, G., 1997. Lipid classes and their content of $n-3$ highly unsaturated fatty acids (HUFA) in *Artemia franciscana* after hatching, HUFA-enrichment and subsequent starvation. Mar. Biol. 130, 81–91.

Dubois, M., Gilles, G.A., Hamilton, J.K., Rebels, P.A., Smith, F., 1956. Colorimetric methods for determination of sugars and related substances. Anal. Chem. 3, 350–356.

Fewster, M.E., Burns, B.J., Mead, J.F., 1969. Quantitative densitometric thin layer chromatography of lipids using cupric acetate reagent. J. Chromatogr. 43, 120–126.

Folch, J., Lees, M., Sloane-Stanley, G.H.S., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497–509.

Fraser, A.J., 1989. Triacylglycerol content as a condition index for fish, bivalve, and crustacean larvae. Can. J. Fish. Aquat. Sci. 46, 1868–1873.

Furuita, H., Takeuchi, T., Toyota, M., Watanabe, T., 1996a. EPA and DHA requirements in early juvenile red sea bream using HUFA enriched *Artemia* nauplii. Fish. Sci. 62, 246–251.

Furuita, H., Takeuchi, T., Watanabe, T., Fujimoto, H., Sekiya, S., Imaizumi, K., 1996b. Requirements of larval yellowtail for eicosapentaenoic acid, docosahexaenoic acid and $n-3$ highly unsaturated fatty acid. Fish. Sci. 62, 372–379.

Habig, W.H., Pabst, M.J., Jacoby, W.B., 1974. Glutathione *S*-transferases. The first enzymatic step in mercaturic acid formation. J. Biol. Chem. 249, 7130–7139.

Hamre, K., Lie, Ø., 1995a. Minimum requirement of vitamin E for Atlantic salmon, *Salmo salar* L., at first feeding. Aquacult. Res. 26, 175–184.

Hamre, K., Lie, Ø., 1995b. α-Tocopherol levels in different organs of Atlantic salmon (*Salmo salar* L.) — effect of smoltification, dietary levels of $n-3$ polyunsaturated fatty acids and vitamin E. Comp. Biochem. Physiol. 111A, 547–554.

Howell, B.R., Tzoumas, T.S., 1991. The nutritional value of *Artemia* nauplii for larval sole *Solea solea* (L.) with respect to their ($n-3$) HUFA content. In: Lavens, P., Sorgeloos, P., Jaspers, E., Ollevier, F. (Eds.), Larvi '91, Fish and Crustacean Larviculture Symposium. European Aquaculture Society, Special Publication no. 15, pp. 63–65.

ICES (International Council for the Exploration of the Sea), 1994. Report of the ICES working group on mass rearing of juvenile marine fish. Int. Counc. Explor. Sea Comm. Meet. (Maricult. Comm.) F: 6.

Izquierdo, M.S., 1996. Essential fatty acid requirements of cultured marine fish larvae. In: Gajardo, G., Coutteau, P. (Eds.), Improvement of the Commercial Production of Marine Aquaculture Species. Proceedings of a workshop on Fish and Mollusc Larviculture. Impresora Creces, Santiago de Chile, pp. 31–44.

Izquierdo, M.S., Watanabe, T., Takeuchi, T., Arakawa, T., Kitajima, C., 1989. Optimal levels in *Artemia* to meet the EFA requirements of red sea bream (*Pagrus major*). In: Takeda, M., Watanabe, T. (Eds.), The Current Status of Fish Nutrition in Aquaculture. Japan Translation Center, Tokyo, Japan, pp. 221–232.

Izquierdo, M.S., Arakawa, T., Takeuchi, T., Haroun, R., Watanabe, T., 1992. Effect of the $n-3$ HUFA levels in *Artemia* on growth of larval Japanese flounder (*Paralichthys olivaceus*). Aquaculture 105, 73–82.

Lavens, P., Sorgeloos, P., Dhert, P., Devresse, B., 1995. Larval foods. In: Bromage, N.R., Roberts, R.J. (Eds.), Broodstock Management and Egg and Larval Quality. Blackwell, London, pp. 373–397.

Lowry, O.H., Roseborough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.

McEvoy, L.A., Navarro, J.C., Bell, J.G., Sargent, J.R., 1995. Autoxidation of oil emulsions during the *Artemia* enrichment process. Aquaculture 134, 101–112.

Merchie, G., 1996. Use of nauplii and metanauplii. In: Lavens, P., Sorgeloos, P. (Eds.), Manual on the Production and Use of Live Food for Aquaculture. FAO Fisheries Technical Paper no. 361, pp. 137–163.

Mourente, G., 1996. Lipid and polyunsaturated fatty acid (PUFA) nutrition and neural system development in early stages of farmed marine fish. In: Gajardo, G., Coutteau, P. (Eds.), Improvement of the Commercial Production of Marine Aquaculture Species. Proceedings of a workshop on Fish and Mollusc Larviculture. Impresora Creces, Santiago de Chile, pp. 23–29.

Mourente, G., Rodríguez, A., Grau, A., Pastor, E., 1998. Utilization of lipids by *Dentex dentex* L. (Osteichthyes, Sparidae) larvae during lecitotrophia and subsequent starvation. Fish Physiol. Biochem., in press.

Niki, E., 1987. Antioxidants in relation to lipid peroxidation. Chemistry and Physics of Lipids 44, 227–253.

Olsen, R.E., Henderson, R.J., 1989. The rapid analysis of neutral and polar lipids using double-development HPTLC and scanning densitometry. J. Exp. Mar. Biol. Ecol. 129, 189–197.

Panchenko, L.F., Brusov, O.S., Gerasimov, A.M., Loktaeva, T.D., 1975. Intramitochondrial localization and release of rat liver superoxide dismutase. FEBS Lett. 55, 84–87.

Pastor, E., Riera, F., Pou, S., Grau, A.M., Grau, A., 1995. Summary of investigations on reproduction and larval rearing of common dentex (*Dentex dentex* L.). ICES Mar. Sci. Symp. 201, 148–152.

Porter, N.A., Caldwell, S.E., Mills, K.A., 1995. Mechanisms of free radical oxidation of unsaturated lipids. Lipids 30, 277–290.

Racker, E., 1955. Glutathione reductase (liver and yeast). In: Colowick, S.P., Kaplan, N.O. (Eds.), Methods in Enzymology, Vol. 2, Academic Press, New York, pp. 722–725.

Rainuzzo, J.R., Reitan, K.I., Olsen, Y., 1997. The significance of lipids at early stages of marine fish: a review. Aquaculture 155, 103–115.

Sargent, J.R., 1995. Origin and functions of egg lipids: nutritional implications. In: Bromage, N.R., Roberts, R.J. (Eds.), Broodstock Management and Egg and Larval Quality. Blackwell, London, pp. 353–372.

Sargent, J.R., Henderson, R.J., Tocher, D.R., 1989. The lipids. In: Halver, J.E. (Ed.), Fish Nutrition, 2nd edn. Academic Press, San Diego, pp. 153–218.

Sargent, J.R., Bell, M.V., Henderson, R.J., Tocher, D.R., 1990. Polyunsaturated fatty acids in marine and terrestrial food webs. In: Mellinger, J. (Ed.), Animal Nutrition and Transport Processes: 1. Nutrition in Wild and Domestic Animals, Vol. 5. Comparative Physiology, Basel, Karger, pp. 11–23.

Sargent, J.R., Bell, M.V., Tocher, D.R., 1993. Docosahexaenoic acid and the development of brain and retina in marine fish. In: Devon, C.A., Baksaas, J., Krokan, H.E. (Eds.), Omega-3 Fatty Acids: Metabolism and Biological Effects. Birkhauser Verlag, Switzerland, pp. 139–149.

Sargent, J.R., Bell, J.G., Bell, M.V., Henderson, R.J., Tocher, D.R., 1995. Requirement criteria for essential fatty acids. J. Appl. Ichthyol. 11, 183–198.

Sargent, J.R., McEvoy, L.A., Bell, J.G., 1997. Requirements, presentation and sources of polyunsaturated fatty acids in marine fish larval fish. Aquaculture 155, 117–127.

Sweetman, J.W., 1992. Larviculture of Mediterranean marine fish species: current status and future trends. J. World Aquacult. Soc. 23, 330–337.

Tocher, D.R., Harvie, D.G., 1988. Fatty acid composition of the major phosphoglycerides from fish neural tissues; $(n-3)$ and $(n-6)$ polyunsaturated fatty acids in rainbow trout (*Salmo gairdneri*) and cod (*Gadus morhua*) brains and retinas. Fish Physiol. Biochem. 5, 229–239.

Tulli, F., Tibaldi, E., 1997. Changes in amino acids and essential fatty acids during early larval rearing of dentex. Aquacult. Int. 5, 229–236.

Watanabe, T., Kiron, V., 1994. Prospects in larval fish dietetics. Aquaculture 124, 223–251.

Wiegand, M.D., 1996. Composition, accumulation and utilization of yolk lipids in teleost fish. Rev. Fish Biol. Fish. 6, 259–286.

Wootton, R.J., 1990. Ecology of Teleost Fishes. Chapman & Hall Fish and Fisheries Series 1, 404 pp.

Yúfera, M., 1982. Morphometric characterization of a small-sized strain of *Brachionus plicatilis* in culture. Aquaculture 27, 277–284.

Zar, J.H., 1984. Biostatistical Analysis, 2nd edn. Prentice-Hall, Englewood Cliffs, NJ.