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Effects of dietary docosahexaenoic acid (22:6n-3) and arachidonic acid (20:4n-6) on the growth, survival, stress resistance and fatty acid composition in black sea bass *Centropristis striata* (Linnaeus 1758) larvae

Troy C Rezek[1], Wade O Watanabe[1], Moti Harel[2] & Pamela J Seaton[3]

[1]Center for Marine Science, University of North Carolina Wilmington, Wilmington, NC, USA

[2]Advanced Bionutrition, Aquanutrition, Columbia, MD, USA

[3]Department of Chemistry, University North Carolina Wilmington, Wilmington, NC, USA

Correspondence: T C Rezek, Center for Marine Science, University North Carolina Wilmington, 601 South College Road, Wilmington, NC 28403 5927, USA. E-mail: rezekt@uncw.edu

Abstract

The objectives of this study were to determine the effects of the dietary docosahexaenoic acid (DHA) to arachidonic acid (ARA) ratio on the survival, growth, hypersaline stress resistance and tissue composition of black sea bass larvae raised from first feeding to metamorphic stages. Larvae were fed enriched rotifers *Brachionus rotundiformis* and *Artemia* nauplii containing two levels of DHA (0% and 10% total fatty acids = TFA) in conjunction with three levels of ARA (0%, 3% and 6% TFA). On d24ph, larvae fed the 10:6 (DHA:ARA) treatment showed significantly ($P < 0.05$) higher survival (62.3%) than larvae fed 0:0 (DHA:ARA) (27.4%). Notochord length and dry weight were also significantly ($P < 0.05$) greater in the 10:6 (DHA:ARA) treatment (8.65 mm, 2.14 mg) than in the 0:0 (DHA:ARA) (7.7 mm, 1.65 mg) treatment. During hypersaline (65 g L^{-1}) challenge, no significant differences ($P > 0.05$) were observed in the median survival time (ST50) between larvae fed 10% DHA (ST50 = 25.6 min) and larvae fed 0% DHA (ST50 = 18.2 min). The results suggested that black sea bass larvae fed prey containing 10% DHA with increasing ARA within the range of 0–6% showed improved growth and survival from first feeding through metamorphic stages.

Keywords: essential fatty acids, arachidonic acid, fish larvae, *Centropristis striata*, growth, survival, hypersaline stress tolerance

Introduction

The nutritional aspects of essential fatty acids (EFA) in fish have been extensively studied, with considerable attention focused on the n-3 polyunsaturated fatty acids (PUFA) requirements of marine fish, particularly eicosapentaenoic acid 20:5n-3 (EPA) and docosahexaenoic acid 22:6n-3 (DHA) (Watanabe, Izquierdo, Takeuchi, Satoh & Kitajima 1989; Takeuchi, Toyota, Satoh & Watanabe 1990; Watanabe 1993; Takeuchi, Masuda, Ishizaki, Watanabe, Kanematsu, Imaizumi & Tsukamoto 1996; Ibeas, Cejas, Fores, Badia, Gomes & Hernandez 1997). The importance of these fatty acids is based on their roles as necessary components of cell membranes, as a major energy source for absorbtion of fat-soluble vitamins (A, D, E and K) and as precursors for prostaglandin hormones. Most marine fish studied to date whose diets are rich in DHA and EPA are usually unable to chain elongate and further desaturate α-linolenic acid 18:3n-3 (ALA) to the more highly unsaturated EPA and DHA (Tacon 1990; Bessonart, Izquierdo, Salhi, Hernandez-Cruz, Gonzalez & Fernandez-Palacios 1999), due to a deficiency in one of two enzymes in the conversion pathway from ALA to EPA, i.e. the C18–C20 elongase multienzyme complex or the Δ5 fatty acid desaturase (Bell & Sargent 2003).

Essential fatty acid enrichment of live feed diets is a routine aspect of larval marine fish aquaculture. In marine fish, both EPA and DHA are considered to be essential because they are required for good growth,

but all marine fish studied to date are barely able to convert ALA to EPA and DHA (Bell & Sargent 2003). To compensate, commercial preparations used to enrich the live food (rotifers and *Artemia* nauplii) typically contain abundant amounts of DHA and lower levels of EPA (Koven, Barr, Lutzky, Ben-Atia, Weiss, Harel, Behrens & Tandler 2001).

In all the marine larval fish species examined to date, DHA has been demonstrated to be superior to EPA in conferring vitality to the larvae (Watanabe 1993). Given that DHA is naturally found at very high levels in neural tissue, it is thought to play a critical role in neural membrane structure and function, including visual acuity for optimum hunting success (Copeman, Parrish, Brown & Harel 2002). Elevated dietary EPA relative to DHA has been shown to have a negative impact on larval neural function and thus growth and survival (Copeman *et al.* 2002). In juvenile turbot *Scophthalmus maximus* (Linnaeus 1758), the most significant aspect of lipid metabolism in the developing brain was the accumulation of DHA, while no other fatty acid increased in percentage in total lipid (Mourente, Tocher & Sargent 1991). Increased mortalities were shown in juvenile turbot when the DHA:EPA ratio in the diet was decreased from 13.8 to 2.2, without changing the percentage of total n-3 PUFA (Bell, Henderson, Pirie & Sargent 1985). Turbot larvae exhibited lower pigmentation success with a lower DHA:EPA ratio in their diet larvae (Rainuzzo, Reiten & Olsen 1997). In starved gilthead seabream *Sparus aurata* (Linnaeus 1758) larvae, DHA was highly conserved, indicating its particular importance during the larval growth stage (Koven, Kissil & Tandler 1989).

Generally, the n-6 series fatty acids are found in a smaller proportion than the n-3 series in marine fish with a ratio of n-3:n-6 PUFA of 10–15:1 (Ackman 1980) and have received little attention in feeding studies in marine fish nutrition. Despite the relatively small amount of arachidonic acid 20:4n-6 (ARA) represented in the tissue, it is also essential for marine carnivorous fish (Koven *et al.* 2001), which are also unable to chain elongate and further desaturate linoleic acid 18:2n-6 (LA) to the more highly unsaturated n-6 fatty acids such as ARA. Enrichment of live foods with lipids rich in n-3 and n-6 fatty acids before feeding to marine fish larvae is necessary for optimal growth, survival and resistance to handling stress (Watanabe 1982; Takeuchi *et al.* 1990; Koven *et al.* 2001).

Among the n-6 PUFA, several studies have pointed out the importance of ARA in fish metabolism. In juvenile turbot, diets containing ARA as the only highly unsaturated fatty acid (HUFA) resulted in higher growth and survival than any mixtures of ARA/DHA or DHA alone (Castell, Bell, Tocher & Sargent 1994). Increasing levels of dietary ARA significantly improved larval growth of the gilthead seabream (Bessonart *et al.* 1999). Survival of the larval gilthead seabream improved when provided with dietary ARA before handling stress (Koven *et al.* 2001). However, levels of ARA that are too high can induce negative effects such as poor larval pigmentation in flatfish species, including yellowtail flounder *Limanda ferruginea* (Storer 1839) (Copeman *et al.* 2002), turbot (Estevez, McEvoy, Bell & Sargent 1998) and halibut *Hippoglossus hippoglossus* (Linnaeus 1758) (McEvoy, Estevez, Bell, Shields, Gara & Sargent 1999).

The black sea bass *Centropristis striata* (Linnaeus 1758) is a commercially and recreationally important marine finfish (family Serranidae), found in the Gulf of Maine to Florida, and is an important candidate for intensive culture (Berlinsky, King, Smith, Hamilton II, Holloway Jr. & Sullivan 1996; Watanabe, Smith, Berlinsky, Woolridge, Stuart, Copeland & Denson 2003). Little is known about the nutritional needs of *C. striata* larvae. The objective of this study was to determine the effects of the DHA:ARA ratio on the survival, growth and hypersaline stress resistance of black sea bass larvae.

Methods

Experimental animals

This study was conducted at the University of North Carolina Wilmington's Center for Marine Science Aquaculture Facility, Wrightsville Beach, NC, USA. Adult black sea bass (mean weight = 0.927 kg; range = 0.320–1.93 kg) were collected in traps set at approximately 14 m depth off Carolina Beach, NC, in November 2000 (Copeland, Watanabe, Carroll & Wheatly 2003). The fish were held outdoors in six cylindrical tanks (diameter = 1.83 m, depth = 0.81 m, volume = 2134 L) under 34 g L^{-1} salinity and a controlled photothermal cycle. Seawater (34 g L^{-1}) was pumped from the Atlantic Intracoastal Waterway adjacent to Wrightsville Beach. In May of 2003, adult females at the post-vitellogenic stage of ovarian development were induced to spawn using leuteinizing hormone releasing hormone-analogue (LHRH-a) (Berlinsky *et al.* 1996; Watanabe *et al.* 2003; Berlinsky, King & Smith 2005) at a dose of 75 μg kg^{-1} body weight. Following volitional spawning, eggs were transferred to a separatory funnel in seawater, where floating (viable) eggs were separated from sinking

(non-viable) eggs. Floating fertilized eggs were placed in 125 L incubators, where their embryonic development was monitored through hatching, approximately 52 h postfertilization. Newly hatched yolksac larvae were transferred to a 15 L separatory funnel before distribution to the rearing aquaria.

Experimental system

The experimental system consisted of four temperature-controlled water baths (152 × 61 × 23 cm), each covered by a light hood (152 × 65 × 19 cm) and surrounded by an opaque black curtain to eliminate extraneous light. Each water bath contained eight cylindrical black plastic aquaria (15 L working volume), where the larvae were reared. Fresh water was continuously re-circulated through the baths and through a heater/chiller to maintain a constant temperature. Illumination was provided by four 40 W fluorescent bulbs supplying full-spectrum lighting simulating natural light.

Experimental design

To study the effects of dietary DHA to ARA ratio on black sea bass larval growth, survival and hypersaline stress resistance, first-feeding stage larvae were fed live prey organisms (rotifers and *Artemia*) that were enriched in emulsions containing different proportions of DHA:ARA. Two levels of DHA (0% and 10%) were used in conjunction with three levels of ARA (0%, 3% and 6%) in a 2 × 3 factorial design. Five replicate aquaria were maintained per treatment.

To begin the experiment, yolksac stage larvae (1 day posthatch = d1ph) were stocked using volumetric methods into aquaria under 34 g L^{-1} and 19 °C and at a density of 40 larvae L^{-1}. Filtered (1 μm) UV-treated seawater was used for larval rearing experiments. The temperature was increased 1 °C day^{-1} until an optimum rearing temperature of 22 °C was reached (Berlinsky, Taylor, Howell Bradley & Theodore 2004).

Live prey enrichment

The DHA:ARA emulsions were formulated by combining different ratios of DHAsco (DHA-rich single cell oil) and ARAsco (ARA-rich single cell oil) with olive oil and water with 5% lecithin, 1% ascorbic acid and 1% Tween-80 (with oil weight) to form a 1:1 oil/water mixture (Advanced Bionutrition, Columbia, MD, USA). Emulsions were sealed and refrigerated for daily use. DHAsco and ARAsco are microbial-derived triglyceride oils that are rich in DHA and ARA respectively. DHAsco was extracted from the heterotrophically grown algae *Crypthecodinium cohnii* and the ARAsco was extracted from the fungus *Mortirella alpina* (Martek Biosciences, Columbia, MD, USA).

Before enrichment, rotifers were cultured in 150 L tanks under continuous illumination, 34 g L^{-1}, and at temperatures of 22–25 °C. Rotifers were fed a diet of preserved microalgae, *Nannochloropsis oculata* (Reed Mariculture, San Jose, CA, USA). *Artemia* were hatched from decapsulated cysts 24 h before enrichment.

During enrichment, rotifers and *Artemia* were held in 3 L beakers in aerated, UV-treated seawater at 34 g L^{-1}. Photoperiod and temperature were maintained at 24 L:0 D and 25 °C. Dissolved oxygen was maintained above 4 ppm. During enrichment, rotifer density was maintained at 500 000 L^{-1}. Enrichment products were added at 0.1 g 500 000 $^{-1}$ rotifers at 0 and 4 h. Rotifers were enriched for 8 h before feeding to the larvae.

During enrichment, *Artemia* density was maintained at 200 000 nauplii L^{-1}. Enrichment products were added at 0.3 g 250 000 $^{-1}$ nauplii at 0 and 8 h. *Artemia* were enriched for 16 h before feeding to the larvae. Samples of rotifers and *Artemia* were collected immediately after enrichment to determine the fatty acid compositions.

Feeding

On d3ph, rotifers were added to the larval rearing units at a density of 10 mL^{-1}. Once feeding began, rotifer density was increased to 20 mL^{-1}. Rotifer density was maintained by quantifying the rotifers in each culture vessel using volumetric methods and adding the appropriate number of enriched rotifers to make up the difference on a daily basis. Background algae *N. oculata* was added daily to maintain a density of 300 000 cells mL^{-1}. On d18ph, newly hatched *Artemia* instar 1 nauplii were added at a density of 0.5 mL^{-1} in addition to enriched rotifers. By d20ph, rotifer feeding stopped and larvae were fed *Artemia* metanauplii enriched with the prescribed treatments at a density of 2 mL^{-1} increasing to 3.5 mL^{-1} by d22ph.

Environmental conditions

Temperature (21.8 ± 0.15 °C), salinity (35 g L^{-1}), dissolved oxygen (6.78 ± 0.08 mg L^{-1}), light intensity

(497 ± 0.74 lx) and pH (7.96 range = 7.85–8.05) were measured daily in each rearing unit. The photoperiod was 18 L:6 D. Aeration was supplied at approximately 80–100 mL min^{-1} through diffusers (4 × 1.3 × 1.3 cm) placed on the bottom at the centre of each aquaria. Once feeding began, 30–55% of water in each rearing unit was exchanged daily, and the water surface of each unit was skimmed three times daily with a paper towel to remove oil films.

Growth and survival

To monitor growth and survival, a minimum of 10 larvae were sampled from each tank on d3, d9, d17 and d24ph using volumetric methods. Larvae were anesthesized (0.3 g L^{-1} 2-phenoxyethanol in freshwater) and then placed in a gridded 100 × 15 mm Petri dish. Living larvae were distinguished from dead ones by the presence or absence of a heartbeat as well as by opacity and appearance.

Notochord length was measured using an ocular micrometer. Wet and dry weights were recorded using a Sartorius (Goettingen, Germany) electrobalance to the nearest 10 μg. Wet weights were recorded by weighing approximately 10 larvae on a microscope slide and then subtracting the weight of the slide. To determine dry weights, larvae were dried at 60 °C until they reached a constant weight (72 h) and then re-weighed.

The larval density (number of larvae L^{-1}) was determined by dividing the number of larvae sampled by the volume of water collected. Larval survival on each sampling date was calculated as the quotient of larval density and larval density determined at d3ph, expressed as a percentage. On d24ph, all the remaining larvae were analysed for carcass fatty acid composition.

Fatty acid analysis

Sample preparations of live prey and larvae for fatty acid analysis were similar to Watanabe *et al.* (1989). Samples were poured onto a 23 μm mesh screen, which was blotted with a sponge from underneath to remove excess water. Samples (approximately 100–150 mg) were then transferred to a 1.2 mL cryogenic vial (Fisher Scientific, Pittsburg, PA, USA), filled with nitrogen and then frozen (– 25 °C) before lipid extraction and fatty acid analysis.

Total lipid and fatty acid analysis was conducted using a revised Folch, Lees and Sloane Stanley (1957) method using a chloroform/methanol mixture (1:1 v/v) to extract lipids from tissue. Total lipid (wet weight) was converted to total lipid dry weight (mg g^{-1}) based on the % dry weight values of larvae and prey of the same treatment groups. Lipid fatty acids were converted to their methyl esters (FAMEs) for GC analysis by the FAME reaction. The first phase of the FAME reaction is a base-catalysed hydrolysis of the glycerol esters, which required the addition of 0.5 M NaOH/MeOH to the sample, which was then heated (70–100 °C) for 30 min. The second phase is an esterification of the free fatty acids catalysed by the addition of 1.5 mL of boron trifluoride-methanol (BF_3) and heating for an additional 30 min. FAMEs from all samples were identified and quantified using GC-FID. Helium was used as the carrier gas. The column temperature profile was: 195 °C, hold for 8 min, ramp to 270 at 15 °C min^{-1} and hold at 270 °C for 2 min. FAME peaks were integrated using the HP CHEMSTATION software package and individual FAMEs were identified by comparison of retention times to standards: GLC-84 (Nu Chek Prep, Elysian, MN, USA), eicosapentaenoic acid methyl ester, arachidonic acid methyl ester (Sigma-Aldrich, St Louis, MO, USA) and stearidonic acid methyl ester (Cayman Chemical, Ann Arbor, MI, USA).

Hypersaline stress resistance

On d24ph larvae were subjected to a hypersaline (65 g L^{-1}) challenge (Dhert, Lavens & Sorgeloos 1991). A minimum of 20 larvae were sampled from each rearing unit and transferred to a 100 × 15 mm Petri dish containing 50 mL of 65 g L^{-1} seawater at 22 °C. Survival was monitored at 5-min intervals until complete mortality was observed. The stress sensitivity index (ST50) was defined as the time at which survival decreased to 50%.

Statistical analysis

The effects of DHA and ARA, and their interaction, on the fatty acid composition, notochord length, dry weight and wet weight were expressed as treatment means ± standard error of the mean (SEM) and compared by using two-way analysis of variance. *F*-max tests were performed on all data sets to test for homogeneity of variances. Treatment means were compared using one-way analysis of variance ANOVA ($P < 0.05$), and a Tukey–Kramer HSD was used for multiple comparisons among means. For percentage data,

arcsine transformation was performed before analysis. Analyses were performed using the JMP IN version 4 statistical software (SAS Institute, Cary, NC, USA).

Results

Fatty acid composition of enriched rotifers

Because no interactive effects were observed between DHA and ARA levels on the fatty acid composition of the enriched rotifers (Table 1), only the main treatment effects are discussed. The n-3 PUFAs were significantly higher ($P<0.05$) in rotifers enriched with 10% DHA [15.3 ± 1.3% total fatty acids (TFA)] than in rotifers enriched with 0% DHA (7.7 ± 0.6% TFA). No significant ($P>0.05$) treatment effects were found for EPA (3.4 ± 0.5% TFA) or docosapentaenoic acid 22:5n-3 (DPA) (1.2 ± 0.2% TFA). The concentration of DHA was higher ($P<0.05$) in rotifers enriched with 10% DHA (7.3 ± 0.9% TFA) than in those enriched with 0% DHA (undetected) (Table 1).

The n-6 PUFAs were higher ($P<0.05$) in rotifers enriched with 6% ARA (10.1 ± 0.4% TFA) than in those enriched with 3% ARA (7.8 ± 0.1% TFA) and 0% (4.5 ± 0.2% TFA). The levels of ARA were significantly higher ($P<0.05$) in rotifers enriched with 6% ARA (5.5 ± 0.5% TFA) than in rotifers enriched with 3% (3.9 ± 0.2% TFA) and 0% ARA (1.1 ± 0.1% TFA).

Fatty acid composition of enriched *Artemia*

No significant ($P<0.05$) effects of DHA and ARA enrichment were observed on the levels of total n-3 PUFA (35.6 ± 3.8% TFA) in enriched *Artemia* (Table 2). No significant ($P>0.05$) treatment effects were observed on the n-3 PUFAs, ALA (27.2 ± 3.3% TFA), stearidonic acid 18:4n-3 (STD) (4.1 ± 0.5% TFA) and EPA (2.4 ± 0.3% TFA). The levels of DHA were higher ($P<0.05$) in *Artemia* enriched with 10% DHA (3.7 ± 0.8% TFA) than in those enriched with 0% DHA (undetected).

The n-6 PUFAs were significantly higher ($P<0.05$) in *Artemia* enriched with 6% ARA (9.5 ± 0.5% TFA) than in those enriched with 3% (8.0 ± 0.2% TFA) and 0% ARA (6.2 ± 0.3% TFA). No significant

Table 1 Total lipid and fatty acid composition (% of total fatty acid) (mean ± SEM, $N = 3$) of enriched rotifers *Brachionus rotundiformis* using six different oil emulsions

	Rotifer treatments (DHA:ARA)					
Fatty acids	**0:0**	**0:3**	**0:6**	**10:0**	**10:3**	**10:6**
14:0	1.5 ± 0.1^{a}	1.4 ± 0.1^{a}	1.5 ± 0.1^{a}	3.9 ± 0.2^{b}	4.0 ± 0.2^{b}	4.5 ± 0.2^{b}
16:0	15.9 ± 0.2	15.8 ± 0.2	16.1 ± 0.5	15.9 ± 0.2	16.5 ± 1.0	17.3 ± 0.9
16:1n-7	5.2 ± 0.2	4.7 ± 0.3	4.7 ± 0.1	5.4 ± 0.4	5.6 ± 0.7	6.2 ± 0.4
18:0	11.8 ± 0.3^{b}	11.0 ± 0.3^{ab}	11.0 ± 0.4^{ab}	9.9 ± 0.3^{a}	10.2 ± 0.3^{a}	10.0 ± 0.4^{a}
18:1n-9	48.4 ± 1.6^{d}	47.8 ± 1.7^{cd}	43.9 ± 0.8^{bcd}	40.8 ± 2.1^{bc}	37.8 ± 0.9^{ab}	32.4 ± 1.5^{a}
18:2n-6	3.5 ± 0.1^{a}	3.7 ± 0.2^{ab}	4.7 ± 0.2^{b}	3.3 ± 0.2^{a}	4.0 ± 0.2^{ab}	4.7 ± 0.4^{b}
18:3n-3	3.3 ± 0.1^{b}	3.2 ± 0.1^{b}	4.1 ± 0.1^{c}	3.3 ± 0.2^{b}	3.0 ± 0.1^{ab}	2.5 ± 0.1^{a}
20:0	2.1 ± 0.5	1.3 ± 0.2	2.3 ± 0.1	1.6 ± 0.3	1.0 ± 0.6	1.2 ± 0.2
20:1	2.6 ± 0.4	3.2 ± 0.5	3.0 ± 0.0	2.1 ± 0.6	2.8 ± 0.9	3.8 ± 1.0
20:4n-6	1.0 ± 0.1^{a}	3.6 ± 0.2^{b}	5.1 ± 0.5^{bc}	1.1 ± 0.1^{a}	4.2 ± 0.2^{b}	5.9 ± 0.5^{c}
20:5n-3	3.5 ± 0.5	3.2 ± 0.6	2.6 ± 0.4	3.9 ± 0.7	3.4 ± 0.3	3.7 ± 0.6
22:5n-3	1.2 ± 0.1	1.1 ± 0.3	1.0 ± 0.1	1.5 ± 0.2	1.3 ± 0.1	1.3 ± 0.2
22:6n-3	Undetected	Undetected	Undetected	7.5 ± 1.0	8.2 ± 0.7	6.4 ± 1.0
ΣSFA	31.2 ± 0.7^{ab}	29.5 ± 0.3^{a}	31.0 ± 0.5^{ab}	31.1 ± 0.3^{ab}	31.6 ± 0.7^{ab}	33.1 ± 0.9^{b}
ΣMFA	56.1 ± 1.2^{d}	55.7 ± 1.2^{d}	51.5 ± 0.9^{cd}	48.3 ± 1.9^{bc}	44.3 ± 0.1^{ab}	42.4 ± 1.0^{a}
Σn-3 PUFA	8.1 ± 0.5^{a}	7.4 ± 0.8^{a}	7.7 ± 0.5^{a}	16.2 ± 1.6^{b}	15.8 ± 0.6^{b}	17.9 ± 1.6^{b}
Σn-6 PUFA	4.6 ± 0.1^{a}	7.3 ± 0.2^{b}	9.7 ± 0.4^{c}	4.4 ± 0.2^{a}	8.2 ± 0.1^{b}	10.5 ± 0.5^{c}
Total lipid (%)	5.9 ± 0.3	6.7 ± 0.3	6.1 ± 0.3	6.2 ± 0.2	5.8 ± 0.5	6.7 ± 0.4
Total lipid (mg g^{-1})	364 ± 25.9	393 ± 14.6	353 ± 16.4	361 ± 13.8	338 ± 28.2	330 ± 22.2

Column titles refer to the percentage of DHA:ARA in each enrichment emulsion. No interactive effects ($P<0.05$) were observed between DHA and ARA levels on the fatty acid composition of the enriched rotifers. Fatty acids in trace amounts (<0.5%) are not reported.

Treatment means were compared using ANOVA ($P<0.05$), and a Tukey–Kramer HSD was used for multiple comparisons among means. Means not sharing a common letter are significantly ($P<0.05$) different.

Total lipid is reported in mg g^{-1} dry weight.

ANOVA, analysis of variance; ARA, arachidonic acid; DHA, docosahexaenoic acid; PUFA, polyunsaturated fatty acids.

Table 2 Total lipid and fatty acid composition (% of total fatty acid) (mean ± SEM, $N = 3$) of enriched *Artemia* using six different oil emulsions

	Artemia treatments (DHA:ARA)					
Fatty acids	**0:0**	**0:3**	**0:6**	**10:0**	**10:3**	**10:6**
14:0	0.7 ± 0.2^{a}	0.9 ± 0.1^{ab}	0.9 ± 0.1^{ab}	2.2 ± 0.1^{c}	1.6 ± 0.2^{bc}	1.9 ± 0.1^{c}
16:0	11.9 ± 0.3	12.0 ± 0.4	12.0 ± 0.3	12.7 ± 0.9	11.7 ± .7	12.7 ± 0.4
16:1n-7	3.6 ± 0.2	3.4 ± 0.3	3.6 ± 0.2	3.7 ± 0.2	3.3 ± .2	3.7 ± 0.3
18:0	7.0 ± 0.4	6.8 ± 0.3	6.9 ± 0.4	6.5 ± 0.2	6.5 ± 0.2	6.4 ± 0.2
18:1n-9	33.3 ± 5.7	35.4 ± 5.5	33.3 ± 3.6	34.3 ± 2.9	31.3 ± 3.5	28.7 ± 1.2
18:2n-6	5.5 ± 0.3	5.7 ± 0.2	5.8 ± 0.4	5.2 ± 0.4	5.5 ± 0.5	5.8 ± 0.3
18:3n-3	29.8 ± 3.7	27.3 ± 4.6	27.3 ± 3.6	25.4 ± 2.4	26.0 ± 3.6	27.3 ± 1.6
18:4n-3	4.5 ± 0.7	4.2 ± 0.7	4.2 ± 0.5	3.9 ± 0.4	3.9 ± 0.4	3.9 ± 0.4
20:4n-6	1.0 ± 0.1^{ab}	2.1 ± 0.3^{bc}	3.5 ± 0.7^{cd}	0.6 ± 0.1^{a}	2.8 ± 0.4^{cd}	3.9 ± 0.2^{d}
20:5n-3	2.2 ± 0.4	2.1 ± 0.4	2.2 ± 0.3	2.4 ± 0.3	2.7 ± 0.1	2.5 ± 0.3
22:6n-3	Undetected	Undetected	Undetected	3.5 ± 0.9	4.4 ± 1.1	3.1 ± 0.4
ΣSFA	20.0 ± 0.4	20.0 ± 0.5	19.8 ± 0.2	21.4 ± 0.8	19.9 ± 0.7	21.0 ± 0.6
ΣMFA	36.9 ± 5.5	38.8 ± 5.4	36.9 ± 3.5	38.0 ± 2.7	34.7 ± 3.3	32.3 ± 1.1
Σn-3 PUFA	36.5 ± 4.8	33.6 ± 5.7	34.0 ± 4.3	35.2 ± 2.8	37.1 ± 3.1	36.9 ± 1.8
Σn-6 PUFA	6.6 ± 0.4^{ab}	7.8 ± 0.2^{bc}	9.3 ± 0.6^{cd}	5.4 ± 0.2^{a}	8.3 ± 0.2^{bcd}	9.7 ± 0.4^{d}
Total lipid (%)	3.7 ± 0.2	3.6 ± 0.6	4.2 ± 0.3	3.8 ± 0.5	4.9 ± 0.8	4.8 ± 0.6
Total lipid (mg g^{-1})	275 ± 17.9	270 ± 45.3	309 ± 19.2	285 ± 33.1	366 ± 60.0	357 ± 47.5

Column titles refer to the percentage of DHA:ARA in each enrichment emulsion. No interactive effects ($P < 0.05$) were observed between DHA and ARA levels on the fatty acid composition of the enriched *Artemia*. Fatty acids in trace amounts (< 0.5%) are not reported.

Treatment means were compared using ANOVA ($P < 0.05$), and a Tukey–Kramer HSD was used for multiple comparisons among means. Means not sharing a common letter are significantly ($P < 0.05$) different.

Total lipid is reported in mg g^{-1} dry weight.

ANOVA, analysis of variance; ARA, arachidonic acid; DHA, docosahexaenoic acid; PUFA, polyunsaturated fatty acids.

($P > 0.05$) treatment effects were found in LA (5.6 ± 0.3% TFA). The concentration of ARA was significantly higher ($P < 0.05$) in *Artemia* enriched with 6% ARA (3.7 ± 0.4% TFA) than in those enriched with 3% (2.5 ± 0.3% TFA) and 0% ARA (0.63 ± 0.2% TFA).

Growth

Because no interactive effects were observed on growth, only the main treatment effects are discussed. No significant ($P > 0.05$) treatment effects were found in notochord length until d17ph (Table 3). On d3ph, notochord length averaged 3.03 ± 0.2 mm among all treatments and it was 4.50 ± 0.1 mm by d9ph. On d17ph, notochord length was higher ($P < 0.05$) in larvae fed 10% DHA (5.82 ± 0.1 mm) than in larvae fed 0% DHA (5.60 ± 0.1 mm). By d24ph, a significant ($P < 0.05$) effect of DHA on notochord length was observed, with no significant ($P > 0.05$) ARA effects. On d24ph, notochord length was higher ($P < 0.05$) in larvae fed 10% DHA (8.55 ± 0.2 mm) than in larvae fed 0% DHA (7.83 ± 0.2 mm). When all means were compared with the 0:0 (DHA:ARA) control (7.70 ± 0.2 mm), notochord length on d24ph was significantly higher ($P < 0.05$) at the 10: 0 (8.61 ± 0.2 mm) and 10: 6 (8.65 ± 0.2 mm) treatment levels.

No significant ($P > 0.05$) treatment effects were found in larval wet and dry weight until d17ph, when significant ($P < 0.05$) effects of DHA and ARA were observed (Table 4). On d3ph, larval wet weight averaged 0.14 ± 0.02 mg larvae^{-1} among all treatments, and by day 9, larval wet weight increased to 0.63 ± 0.04 mg larvae^{-1}. On d17ph, larval wet weight was higher ($P < 0.05$) in larvae fed 10% DHA (3.10 ± 0.1 mg) than in larvae fed 0% DHA (2.61 ± 0.2 mg). Also on d17ph, larval wet weight was higher ($P < 0.05$) in larvae fed 6% ARA (3.20 ± 0.1 mg) than in larvae fed 0% or 3% ARA (2.69 ± 0.1 mg). When all the means were compared with the 0: 0 (DHA:ARA) control (2.45 ± 0.1 mg), wet weight was significantly higher ($P < 0.05$) in the 10:6 (3.53 ± .05 mg) treatment level (Table 4). By d24ph, inter-replicate variability was high, and no significant ($P > 0.05$) treatment effects on larval wet weight were observed.

Table 3 Notochord length (mm) (mean ± SEM, $N = 10$) of larvae fed enriched rotifers *Brachionus rotundiformis* and *Artemia*, using six different oil emulsions consisting of varying levels of DHA (0%, 10%) to ARA (0%, 3%, 6%)

dph	0:0	0:3	0:6	10:0	10:3	10:6
3	3.0 ± 0.1	2.9 ± 0.2	3.1 ± 0.1	3.1 ± 0.1	3.0 ± 0.1	3.1 ± 0.1
9	4.6 ± 0.1	4.4 ± 0.1	4.5 ± 0.1	4.4 ± 0.1	4.4 ± 0.0	4.6 ± 0.1
17	5.6 ± 0.1	5.6 ± 0.1	5.7 ± 0.1	5.8 ± 0.1	5.9 ± 0.1	5.7 ± 0.1
24	7.7 ± 0.2^{a}	7.8 ± 0.2^{a}	8.0 ± 0.2^{ab}	8.6 ± 0.2^{b}	8.4 ± 0.2^{ab}	8.7 ± 0.2^{b}

No interactive effects ($P < 0.05$) were observed between DHA and ARA levels on larval notochord length.
Treatment means were compared using ANOVA ($P < 0.05$), and a Tukey–Kramer HSD was used for multiple comparisons among means. Means not sharing a common letter are significantly ($P < 0.05$) different.
ANOVA, analysis of variance; ARA, arachidonic acid; DHA, docosahexaenoic acid.

Table 4 Wet weight (mg) (mean ± SEM, $N = 10$) of larvae fed enriched rotifers *Brachionus rotundiformis* and *Artemia*, using six different oil emulsions consisting of varying levels of DHA (0%, 10%) to ARA (0%, 3%, 6%)

dph	0:0	0:3	0:6	10:0	10:3	10:6
3	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
9	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.1	0.6 ± 0.0	0.7 ± 0.0	0.7 ± 0.0
17	2.5 ± 0.1^{a}	2.5 ± 0.3^{a}	2.9 ± 0.2^{ab}	3.0 ± 0.2^{ab}	2.8 ± 0.1^{ab}	3.5 ± 0.1^{b}
24	10.4 ± 1.5	11.1 ± 2.4	11.9 ± 1.3	11.2 ± 1.2	13.2 ± 2.6	11.3 ± 2.4

No interactive effects ($P < 0.05$) were observed between DHA and ARA levels on larval wet weight.
Treatment means were compared using ANOVA ($P < 0.05$), and a Tukey–Kramer HSD was used for multiple comparisons among means. Means not sharing a common letter are significantly ($P < 0.05$) different.
ANOVA, analysis of variance; ARA, arachidonic acid; DHA, docosahexaenoic acid.

Table 5 Dry weight (mg) (mean ± SEM, $N = 10$) of larvae fed enriched rotifers *Brachionus rotundiformis* and *Artemia*, using six different oil emulsions consisting of varying levels of DHA (0%, 10%) to ARA (0%, 3%, 6%)

dph	0:0	0:3	0:6	10:0	10:3	10:6
3	0.01 ± 0.0	0.02 ± 0.0	0.02 ± 0.0	0.03 ± 0.0	0.03 ± 0.0	0.02 ± 0.0
9	0.04 ± 0.0	0.06 ± 0.0	0.05 ± 0.0	0.04 ± 0.0	0.06 ± 0.0	0.06 ± 0.0
17	0.4 ± 0.0^{a}	0.4 ± 0.0^{a}	0.5 ± 0.0^{ab}	0.5 ± 0.1^{ab}	0.5 ± 0.0^{ab}	0.6 ± 0.0^{b}
24	1.7 ± 0.3	1.5 ± 0.1	1.9 ± 0.2	1.4 ± 0.7	2.1 ± 0.4	2.1 ± 0.4

No interactive effects ($P < 0.05$) were observed between DHA and ARA levels on larval dry weight.
Treatment means were compared using ANOVA ($P < 0.05$), and a Tukey–Kramer HSD was used for multiple comparisons among means. Means not sharing a common letter are significantly ($P < 0.05$) different.
ANOVA, analysis of variance; ARA, arachidonic acid; DHA, docosahexaenoic acid.

On d3ph, dry weight averaged 0.02 ± 0.003 mg larvae^{-1} among all treatments and, by d9ph, dry weight increased to 0.05 ± 0.01 mg larvae^{-1}. A significant ($P < 0.05$) effect of DHA on dry weight was observed by d17ph, while there were no significant ($P > 0.05$) ARA effects (Table 5). On d17ph, dry weight was higher ($P < 0.05$) in larvae fed 10% DHA (0.55 ± 0.03 mg) than in larvae fed 0% DHA (0.45 ± 0.04 mg). When all the means were compared with the 0: 0 (DHA:ARA) control (0.43 ± 0.03 mg), dry weight was higher ($P < 0.05$) at the 10:6 (0.62 ± 0.02 mg) treatment level (Table 5). By d24ph, the inter-replicate variability was high, and no significant ($P > 0.05$) treatment effects were observed on larval dry weight.

Survival

On d9ph, the mean survival for all treatments was 85.0 ± 6.8%, with no significant ($P > 0.05$) treatment effects (Table 6). On d17ph, the mean survival for all treatments decreased to 72.4 ± 9.4%, with no significant ($P > 0.05$) treatment effects. By d24ph, a signifi-

Table 6 Survival (mean ± SEM, $N = 5$) from d3ph of post-metamorphic black sea bass larvae (d24ph) fed enriched rotifers and *Artemia* with different of DHA (0%, 10%) and ARA (0%, 3%, 6%)

dph	0:0	0:3	0:6	10:0	10:3	10:6
9	95.1 ± 4.9	86.0 ± 6.3	85.7 ± 9.2	80.2 ± 5.5	82.9 ± 7.3	80.1 ± 7.6
17	85.9 ± 7.9	65.4 ± 11.5	67.0 ± 10.4	71.0 ± 5.8	76.3 ± 9.0	68.8 ± 11.7
24	27.4 ± 6.5^{a}	34.6 ± 14.3^{ab}	30.4 ± 4.7^{ab}	46.2 ± 6.8^{ab}	53.9 ± 11.5^{ab}	62.3 ± 13.9^{b}

No interactive effects ($P < 0.05$) were observed between DHA and ARA levels on larval survival.
Treatment means were compared using ANOVA ($P < 0.05$), and a Tukey–Kramer HSD was used for multiple comparisons among means. Means not sharing a common letter are significantly ($P < 0.05$) different.
ANOVA, analysis of variance; ARA, arachidonic acid; DHA, docosahexaenoic acid.

cant ($P < 0.05$) effect of DHA on survival was observed, while there were no significant ($P > 0.05$) ARA effects. On d24ph, survival was higher ($P < 0.05$) in larvae fed 10% DHA (54.1 ± 10.7%) than in larvae fed 0% DHA (30.8 ± 8.5%). When all the means were compared with the 0: 0 (DHA:ARA) control (27.4 ± 6.5%), survival was significantly higher ($P \leq 0.05$) at the 10: 6 (62.3 ± 13.9%) treatment level (Table 6).

Hypersaline stress evaluation

No significant ($P > 0.05$) treatment effects were found in ST50 following hypersaline (65 g L^{-1}) exposure. ST-50 appeared to be higher ($0.05 < P < 0.10$) in the 10% DHA (25.6 ± 13.2 min) than in the 0% DHA (18.2 ± 5.4 min) treatments.

Fatty acid composition of d24ph larvae

The n-3 PUFAs were higher ($P < 0.05$) in larvae fed 10% DHA (35.9 ± 0.8% TFA) than in larvae fed 0% DHA (33.2 ± 0.7% TFA) (Table 7). The n-3 PUFA levels were also higher ($P < 0.05$) in larvae fed 0% ARA (35.3 ± 0.7% TFA) than in larvae fed 3% (34.5 ± 0.8% TFA) and 6% (32.8 ± 0.7% TFA) ARA. The concentration of ALA and STD was higher ($P < 0.05$) in larvae fed 0% DHA (22.2 ± 0.5%, 3.2 ± 0.1% TFA) than in larvae fed 10% DHA (21.1 ± 0.6%, 3.0 ± 0.1% TFA). STD levels were higher ($P < 0.05$) in larvae fed 0% ARA (3.3 ± 0.1% TFA) than in larvae fed 3% (2.9 ± 0.4% TFA) and 6% (3.1 ± 0.9% TFA) ARA. No significant ($P > 0.05$) differences were found in DPA (0.88 ± 0.1% TFA) (Table 8). The concetration of EPA was the highest ($P < 0.05$) in larvae fed 10% DHA (5.1 ± 0.2% TFA) and 0% ARA (5.2 ± 0.1% TFA) (Table 7). DHA levels were higher ($P < 0.05$) in larvae fed 10% DHA (5.8 ± 0.5% TFA) than in larvae fed 0% DHA (2.4 ± 0.2% TFA).

The n-6 PUFAs were higher ($P < 0.05$) in larvae fed 6% ARA (14.0 0.4% TFA) than in larvae fed 3% (12.1 ± 0.2% TFA) and 0% ARA (9.5 ± 0.3% TFA) (Table 8). No significant ($P > 0.05$) treatment effects were found in LA (7.8 ± 0.2% TFA). The concentration of ARA was higher ($P < 0.05$) in larvae fed 6% ARA (6.0 ± 0.3% TFA) than in larvae fed 3% (4.2 ± 0.2% TFA) and 0% ARA (1.9 ± 0.05% TFA).

Discussion

While slightly lower than prescribed, the DHA levels in rotifers increased with increasing levels in their diets. Rotifers enriched with 0% DHA contained no detectable DHA, while rotifers enriched with 10% DHA contained an average of 7.3% DHA. Other workers have reported a direct relationship between PUFA levels in rotifers and levels in their enriched diets (Watanabe, Kitajima & Fujita 1983; Rainuzzo, Olsen & Rosenlund 1989; Frolov, Pankov, Geradze, Pankova & Spektorova 1991).

While EPA was not supplied in the enrichment emulsions, a small percentage (mean = 3.4%) was found in rotifers in all the treatment groups. EPA was probably derived from their diet of *N. oculata*, which is naturally high (31.4%) in EPA (Reed Mariculture, Campbell, CA, USA).

The ARA levels found in rotifers were similar to the levels provided in their diets. ARA levels in enriched rotifers ranged from 1% to 5% at treatment levels ranging from 0% to 6%. The small amount of ARA (1%) found in rotifers enriched with 0% ARA can also be attributed to their diet of *N. oculata*, which contains 3.9% ARA (Reed Mariculture, Campbell, CA, USA).

Artemia enriched with 0% DHA contained no detectable DHA, while *Artemia* enriched with 10% DHA contained only 3.7% DHA (range = 3.1–4.4%). Unlike rotifers, *Artemia* enrichment is difficult due to

Table 7 Fatty acid composition (% of total fatty acid) (mean ± SEM, $N = 3$) of larvae fed enriched rotifers *Brachionus rotundiformis* and *Artemia*, using six different oil emulsions consisting of varying levels of DHA (0%, 10%) to ARA (0%, 3%, 6%)

	Enriched diets received by larvae (DHA:ARA)					
Fatty acids	**0:0**	**0:3**	**0:6**	**10:0**	**10:3**	**10:6**
14:0	0.1 ± 0.1	0.4 ± 0.2	0.2 ± 0.1	0.6 ± 0.2	0.8 ± 0.0	0.6 ± 0.2
16:0	13.4 ± 0.2	13.8 ± 0.2	13.7 ± 0.3	14.1 ± 0.5	14.4 ± 0.3	14.4 ± 0.3
16:1n-7	2.7 ± 0.3	2.7 ± 0.3	2.5 ± 0.2	2.9 ± 0.1	2.7 ± 0.1	2.6 ± 0.1
18:0	8.1 ± 0.2 ^{c}	7.5 ± 0.2^{b}	7.5 ± 0.1 ^{b}	7.2 ± 0.1^{b}	6.6 ± 0.1^{a}	6.7 ± 0.0^{a}
18:1n-9	28.4 ± 0.2^{d}	27.0 ± 0.2^{dc}	26.1 ± 0.6^{cb}	25.1 ± 0.7^{cb}	23.9 ± 0.5^{ba}	22.6 ± 0.6^{a}
18:2n-6	7.4 ± 0.2	7.7 ± 0.1	7.7 ± 0.3	7.7 ± 0.4	8.0 ± 0.3	8.1 ± 0.3
18:3n-3	22.6 ± 0.1	21.8 ± 0.5	22.1 ± 0.8	22.2 ± 0.5	20.2 ± 0.3	20.7 ± 1.0
18:4n-3	3.4 ± 0.1^{b}	3.2 ± 0.1 ^{ba}	3.0 ± 0.1^{ba}	3.2 ± 0.2^{ba}	2.9 ± 0.1^{ba}	2.8 ± 0.1^{a}
20:0	1.3 ± 0.0	1.0 ± 0.3	0.9 ± 0.3	1.1 ± 0.1	1.1 ± 0.1	0.9 ± 0.2
20:1	2.6 ± 0.0^{ba}	2.5 ± 0.0^{ba}	2.6 ± 0.3^{ba}	2.5 ± 0.1^{ba}	2.3 ± 0.0^{a}	3.1 ± 0.4^{b}
20:4n-6	2.0 ± 0.0 ^{a}	3.9 ± 0.3^{b}	6.3 ± 0.3^{c}	1.8 ± 0.1^{a}	4.5 ± 0.1^{b}	5.8 ± 0.3^{c}
20:5n-3	4.8 ± 0.1^{ba}	4.7 ± 0.1^{ba}	4.2 ± 0.1^{a}	5.5 ± 0.2^{c}	5.1 ± 0.2^{cb}	4.7 ± 0.1^{ba}
22:5n-3	1.0 ± 0.1	0.75 ± 0.2	0.87 ± 0.0	0.69 ± 0.2	0.88 ± 0.0	1.1 ± 0.1
22:6n-3	2.0 ± 0.1^{a}	2.9 ± 0.1^{a}	2.2 ± 0.4^{a}	5.2 ± 0.2^{b}	6.5 ± 0.4^{b}	5.7 ± 0.8^{b}
ΣSFA	22.9 ± 0.3	22.7 ± 0.5	22.3 ± 0.4	23.0 ± 0.3	22.8 ± 0.4	22.6 ± 0.4
ΣMFA	33.8 ± 0.3^{c}	32.3 ± 0.2^{cb}	31.3 ± 0.6^{b}	30.5 ± 0.6^{ba}	28.9 ± 0.6^{a}	28.4 ± 0.7^{a}
Σn-3 PUFA	33.8 ± .03^{ba}	33.4 ± 0.7^{ba}	32.4 ± 1.0^{a}	36.8 ± 1.1^{b}	35.7 ± 0.8^{ba}	35.0 ± 0.5^{ba}
Σn-6 PUFA	9.4 ± 0.2^{a}	11.6 ± 0.3^{b}	13.9 ± 0.3^{c}	9.5 ± 0.3^{a}	12.6 ± 0.2^{cb}	14.0 ± 0.5^{c}
Total lipid (%)	4.6 ± 0.8	4.2 ± 0.6	3.6 ± 0.7	4.6 ± 0.1	5.1 ± 0.2	3.4 ± 0.9
Total lipid (mg g^{-1})	265 ± 46.0	275 ± 38.5	211 ± 43.7	264 ± 7.6	293 ± 8.9	195 ± 53.8

Column titles refer to the percentage of DHA:ARA in each enrichment emulsion. No interactive effects ($P < 0.05$) were observed between DHA and ARA levels on fatty acid composition of the black sea bass larvae. Fatty acids in trace amounts (<0.5%) are not reported.

Treatment means were compared using ANOVA ($P < 0.05$), and a Tukey–Kramer HSD was used for multiple comparisons among means. Means not sharing a common letter are significantly ($P < 0.05$) different.

Total lipid is reported in mg g^{-1} dry weight.

ANOVA, analysis of variance; ARA, arachidonic acid; DHA, docosahexaenoic acid; PUFA, polyunsaturated fatty acids.

their rapid development and catabolism of DHA (Sorgeloos, Dhert & Candreva 2001). Higher levels of DHA and ARA in *Artemia* could be achieved if the enrichment period is extended from 16 h (as used in this study) to 19–24 h. (McEvoy, Navarro, Bell & Sargent 1995). Enrichment success in *Artemia* also depends on genetic characteristics. Chinese strains of *Artemia* accumulated higher levels of DHA than the Great Salt Lake strain or the San Francisco Bay strain used in this study (Dhert, Sorgeloos & Devresse 1993).

In the 0:0 treatment, ARA (0.23%) levels were similar to the levels found in newly hatched unenriched *Artemia franciscana* (0.8% TFA) (Coutteau & Mourente 1997). However, the ARA level in *Artemia* enriched with 6% ARA was only 3.9%, suggesting poor uptake. It is possible that the *Artemia* enrichment period (16 h) used was inadequate for optimal uptake of DHA and ARA from the emulsions. Other workers have reported that an 18-h enrichment period produced ARA levels in *Artemia* more closely reflecting their diets (Estevez *et al.* 1998).

In this study, growth (body length and body weight) was higher in larvae fed prey enriched with 10% DHA (vs. 0% DHA), with the fastest growth at the 10:6 (DHA:ARA) treatment level. Red sea bream *Pagrus major* (Temminck and Schlegel 1843) larvae fed rotifers enriched with $\geq$ 20% DHA grew faster than those fed rotifers enriched with $\leq$ 2% DHA (Watanabe *et al.* 1989). Improved growth with increasing levels of dietary DHA was also reported in larval yellowtail *Seriola quinqueradiata* (Temminck and Schlegel 1843) (Furuita, Takeuchi, Watanabe, Fujimoto, Sekiya & Imaizumi 1996; Copeman *et al.* 2002) and larval striped jack (Takeuchi *et al.* 1996). Juvenile herring *Clupea harengus* (Linnaeus 1758) (Bell, Batty, Dick, Fretwell, Navarro & Sargent 1995) fed unenriched *Artemia* nauplii containing no DHA during the period of rod development showed impaired visual performance. Maximum visual acuity at the first feeding larval stage presumably increases hunting success and growth.

Black sea bass larvae fed the 10:6 (DHA:ARA) diet grew significantly faster than larvae fed the control

diet of 0:0 (DHA:ARA). In 21–32-day-old gilthead seabream, growth was faster in larvae fed 6.3% ARA vs. 1.5% (TFA) (Van Anholt, Koven, Lutzky & Wendelaar Bonga 2004). In contrast to these findings, dietary ARA within the range of 0–15% TFA had no effect on the growth of larval gilthead seabream (Koven *et al.* 2001) or of larval Japanese flounder *Paralichthys olivaceus* (Temminck and Schlegel 1843) (Estevez, Kaneko, Seikai, Tagawa & Tanaka 2001). In larval white bass *Morone chrysops* (Rafinesque 1820), dietary ARA levels of 0–26% TFA also had no effect on growth. On the other hand, in striped bass *Morone saxatilis* (Walbaum 1792) the fastest growth was found at intermediate ARA levels (13% TFA) within a range of 0–26% ARA (Harel & Place 2003). These congeneric differences were suggested to reflect the freshwater origin of white bass and marine origin of striped bass (Harel & Place 2003). Freshwater omnivores convert LA to ARA, while carnivorous marine fish cannot synthesize ARA, emphasizing the importance of dietary ARA supplementation (Sargent 1995).

In agreement with growth data, survival on d24ph was higher in larvae fed 10% DHA (55%) than in those fed 0% DHA (31%). Increased survival of larvae fed diets containing higher levels of DHA was reported in red sea bream (21.8% TFA) (Watanabe *et al.* 1989), yellowtail (43.3% TFA) (Copeman *et al.* 2002) and larval striped jack (9.1% TFA) (Takeuchi *et al.* 1996).

Survival on d24ph was better in larvae fed the 10:6 (DHA:ARA) (62%) than in those fed the control diet of 0:0 (DHA:ARA) (27%). Larval summer flounder *Paralichthys dentatus* (Linnaeus 1766) fed rotifers and *Artemia* enriched to contain from 0% to 12% ARA showed increased growth and survival to d45ph at ARA levels up to 6%, with no further improvement at higher levels (Willey, Bengtson & Harel 2003). Survival of the gilthead seabream (d35ph) fed diets containing from 0% to 5.3% ARA (dry weight) was the highest at 1.8% (Bessonart *et al.* 1999) and 1.7% (Koven *et al.* 2001). These studies suggested that dietary ARA during the rotifer feeding stage significantly improved larval survival through the metamorphic stages (Koven *et al.* 2001; Willey *et al.* 2003), but with species-specific differences in the absolute ARA requirement.

In this study, larvae fed the 10% DHA treatments showed only a marginally higher stress resistance ($0.05 < P < 0.10$) than those fed 0% DHA. In comparison, Japanese flounder *P. olivaceus* (Temminck and Schlegel 1846) (Furuita, Konishi & Takeuchi 1999), milkfish *Chanos chanos* (Forsskal 1775) (Gapasin, Bombeo, Lavens, Sorgeloos & Nelis 1998) and red drum *Sciaenops ocellatus* (Linnaeus 1776) (Brinkmeyer & Holt 1998) larvae fed high DHA showed significantly increased resistance when exposed to a hypersaline challenge of 65–70 g L^{-1}. ARA is readily incorporated into cell membrane phospholipids and is a precursor to the formation of eicosanoids (e.g. prostaglandins) in fish and mammals (Sargent, Bell, McEvoy, Tocher & Estevez 1999). In response to stress, prostaglandins (primarily PGE_2) stimulate cortisol production in the hypothalamus–pituitary–adrenal axis in mammals (Lands 1991; Hockings, Grice, Crosby, Walters, Jackson & Jackson 1993; Nye, Hockings, Grice, Torpy, Walters, Crosbie, Wagenaar, Cooper & Jackson 1997) and possibly in the hypothalamus–pituitary–interrenal axis in fish (Gupta, Lahlou, Botella & Porthe-Nibelle 1985; Koven 2003). Cortisol promotes chloride cell differentiation and Na^+K^+ ATPase activity in the gills (Koven 2003), allowing adaptation to different salinities (Harel, Gavasso, Leshin, Gubernatis & Place 2001). Therefore, resistance to salinity stress in marine fish would be expected to improve at increased ARA levels (Bell & Sargent 2003).

In this study, no significant effects of dietary ARA on hypersaline stress resistance in black sea bass larvae were observed. In contrast, larval summer flounder fed a diet of 6% ARA (TFA) showed increased stress resistance when exposed to 70 g L^{-1} on d18ph and to 80 g L^{-1} on d45ph (Willey *et al.* 2003). In gilthead sea bream, larvae fed dietary ARA (0.59–5.86% dry weight) showed improved survival when subjected to acute handling stress (Koven 2003). It is possible that the acute hypersaline conditions (65 g L^{-1}) used in the present study were too high to allow the effects of diet on osmoregulation to be resolved. Lower or fluctuating salinity, analysis of Na^+K^+ ATPase gene expression or cortisol production analysis may provide better resolution of the effects of dietary DHA or ARA on resistance to salinity stress (Jensen, Madsen & Kristiansen 1998; Koven 2003; Van Anholt *et al.* 2004).

On d24ph, the DHA levels in larvae fed 10% DHA (5.8% wet weight) were lower than the levels provided in enriched rotifers (7.4% wet weight). This was attributed to the relatively low DHA levels (3.4%) in *Artemia* fed after d18ph. Low DHA levels (2.4%) were found in larvae fed 0% DHA, and EPA levels were higher in larvae fed 10% DHA (EPA = 5.1%) than in larvae fed 0% DHA (EPA = 4.6%). Because all the enrichment emulsions were devoid of EPA, the conversion of EPA to DHA in black seabass larvae fed 0% DHA is suggested. Similar findings were reported

in juvenile turbot (Linares & Henderson 1991) and gilthead seabream (Mourente & Tocher 1994).

Carcass ARA levels on d24ph were higher than the levels provided in enriched rotifers and were nearly twice the levels provided in enriched *Artemia*. This suggests that dietary ARA (orginating from *N. oculata* fed to rotifers) was accumulated to levels well above those provided in their diet, as reported in turbot (Linares & Henderson 1991) and in larval mahi mahi *Coryphaena hippurus* (Linnaues 1758) (Ako, Kraul & Tamaru 1991). As seen in gilthead seabream larvae (Bessonart *et al*. 1999), tissue levels of ARA in black sea bass larvae increased with increasing dietary levels, suggesting that larval requirements for ARA can be met through dietary manipulations.

Similar to gilthead seabream and turbot (Linares & Henderson 1991; Mourente & Tocher 1994), the levels of ALA and LA in black sea bass larvae on d24ph were higher than the levels provided in enriched rotifers and *Artemia*, suggesting that these EFA precursors were accumulated, but not converted to the longer chain unsaturated fatty acids, demonstrating a dietary requirement for 20 and 22 carbon EFAs.

In summary, a DHA level of 10% with increasing ARA within the range of 0–6% improved the growth and survival of larval black sea bass, but with only marginal effects on hypersaline stress resistance when exposed to an acute salinity challenge. On d24ph, the ARA concentrations in larvae were higher than the levels provided in their diets, suggesting accumulation of ARA in larval tissues and indicating a high requirement for this fatty acid. Future studies investigating higher DHA and ARA levels are warranted.

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References

Ackman R.G. (1980) Fish lipids. Part 1. In: *Advances in Fish Science and Technology*. (ed. by Connell J. J.), pp. 86–103. Fishing News Books, Farnham, Surrey, UK.

Ako H., Kraul S. & Tamaru C. (1991) *Pattern of Fatty Acid Loss in Several Warmwater Fish Species During Early Development. Larvi 91 Fish and Crustacean Larivculture Symposium. Special Publication No. 15*. European Aquaculture Society, Gent, Belgium, pp. 23–25.

Bell J.G. & Sargent J.R. (2003) Arachidonic acid in aquaculture feeds: current status and future opportunities. *Aquaculture* **218**, 491–499.

Bell M.V., Henderson R.J., Pirie B.J.S. & Sargent J.R. (1985) Effects of dietary polyunsaturated fatty acids deficiencies on mortality, growth and gill structure in the turbot, *Schopthalmus maximus*. *Journal of Fish Biology* **26**, 181–191.

Bell M.V., Batty R.S., Dick J.R., Fretwell K., Navarro J.C. & Sargent J.R. (1995) Dietary defiency of docosahexaenoic acid impairs vision at low light intensities in juvenile herring *Clupea harengus* L. *Lipids* **30**, 443–449.

Berlinsky D.L., King W.V., Smith T.I.J., Hamilton R.D. II, Holloway J. Jr & Sullivan C.V. (1996) Induced ovulation of Southern flounder *Paralichthys lethostigma* using gonadotropin releasing hormone analogue implants. *Journal of the World Aquaculture Society* **27**, 143–152.

Berlinsky D.L., Taylor J.C., Howell R., Bradley T.M., Theordore I.J. (2004) The effects of temperature and salinity on early life stages of black sea bass *Centropristis striata*. *Journal of the World Aquaculture Society* **35**, 335–344.

Berlinsky D.L., King W.V. & Smith T.I.J. (2005) The use of luteinizing hormone releasing hormone analogue for ovulation induction in black sea bass *Centropristis striata*. *Aquaculture* **250**, 813–822.

Bessonart M., Izquierdo M.S., Salhi M., Hernandez-Cruz C.M., Gonzalez M.M. & Fernandez-Palacios H. (1999) Effect of dietary arachidonic acid levels on growth and survival of gilthead seabream *Sparus aurata* L. *larvae*. *Aquaculture* **179**, 265–275.

Brinkmeyer R.L. & Holt G.J. (1998) Highly unsaturated fatty acids in diets for red drum *Sciaenops ocellatus* larvae. *Aquaculture* **161**, 253–268.

Castell J.D., Bell J.G., Tocher D.R. & Sargent J.R. (1994) Effects of purified diets containing different combinations of arachidonic and docosahexaenoic acid on survival, growth and fatty acid composition of juvenile turbot *Scophthalmus maximus*. *Aquaculture* **128**, 315–333.

Copeland K.A., Watanabe W.O., Carroll P.M. & Wheatly S.K. (2003) Growth and feed utilization of captive wild black sea bass *Centropristis striata* at four different densities in a recirculating tank system. *Journal of the World Aquaculture Society* **34**, 300–307.

Copeman L.A., Parrish C.C., Brown J.A. & Harel M. (2002) Effects of docosahexanoic, eicosapentaenoic, and arachidonic acids on the early growth, survival, lipid composition and pigmentation of yellowtail flounder *Limanda ferruginea*: a live food enrichment experiment. *Aquaculture* **210**, 285–304.

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

Dhert P., Lavens P. & Sorgeloos P. (1991) *Stress Evaluation: A Tool for Quality Control of Hatchery Produced Shrimp and Fish fry.*. Laboratory of Aquaculture and Artemia Reference Center, Gent, Belgium.

Dhert P., Sorgeloos P. & Devresse B. (1993) Contributions towards a specific DHA enrichment in the live food *Brachionus plicatilis* and *Artemia* sp. In: *Proceedings of the 1st International Conference on Fish Farming Technology*, Trondheim, Norway, 9–12 August 1993 (ed. by H. Reinertsen, L.A. Dahle, L. Jorgenson & K. Tvinnerein), pp. 109–114. AA Balkema, Rotterdam, the Netherlands.

Estevez A., McEvoy L.A., Bell J.G. & Sargent J.R. (1998) Effects of temperature and starvation time on the pattern and rate of loss of essential fatty acids in *Artemia* nauplii previously enriched using arachidonic acid and eicosapentaenoic acid-rich emulsions. *Aquaculture* **165**, 295–311.

Estevez A., McEvoy L.A., Bell J.G. & Sargent J.R. (1999) Growth, survival, lipid composition and pigmentation of turbot *(Scophtalmus maximus)* larvae fed live-prey enriched in Archidonic and Eicosapentaenoic acids. *Aquaculture* **180**, 321–343.

Estevez A., Kaneko T., Seikai T., Tagawa M. & Tanaka M. (2001) ACTH and MSH production in Japanese flounder *Paralichthys olivaceus* larvae fed arachidonic acid enriched live prey. *Aquaculture* **192**, 309–319.

Folch J., Lees M. & Sloane Stanley G.H. (1957) A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* **226**, 497–509.

Frolov A.V., Pankov S.L., Geradze K.N., Pankova S.A. & Spektorova L.V. (1991) Influence of the biochemical composition of food on the biochemical compostition of the rotifer *Brachionus plicatilis*. *Aquaculture* **97**, 181–202.

Furuita H., Takeuchi T., Watanabe T., Fujimoto H., Sekiya S. & Imaizumi K. (1996) Requirements of larval yellowtail for eicosapentaenoic acid, docosahexaenoic acid, and highly unsaturated fatty acid. *Fisheries Science* **62**, 372–379.

Furuita H., Konishi K. & Takeuchi T. (1999) Effect of different levels of eicosapentaenoic acid and docosahexaenoic acid in Artemia nauplii on growth, survival and salinity tolerance of larvae of the Japanese flounder, *Paralichthys olivaceus*. *Aquaculture* **170**, 59–69.

Gapasin R.S.J., Bombeo R., Lavens P., Sorgeloos P. & Nelis H. (1998) Enrichment of live food with essential fatty acids and vitamin C: effects of milkfish *Chanos chanos* larval performance. *Aquaculture* **162**, 269–286.

Gupta O.P., Lahlou B., Botella J. & Porthe-Nibelle J. (1985) In vivo and in vitro studies on the release of cortisol from interrenal tissue in trout: 1. Effects of ACTH and prostaglandins. *Experimental Biology* **43**, 201–212.

Harel M. & Place A.R. (2003) Tissue essential fatty acid compostion and competitive response to dietary manipulations in white bass *Morone chrysops*, striped bass (*M. saxatilis*) and hybrid striped bass *M. chrysops x M. saxatilis*. *Comparative Biochemistry and Physiology Part B* **135**, 83–94.

Harel M., Gavasso S., Leshin J., Gubernatis A. & Place A.R. (2001) The effect of tissue docosahexaenoic and arachidonic acids levels on hypersaline tolerance and leucocyte composition in striped bass *Morone saxatilis* larvae. *Fish Physiology and Biochemistry* **24**, 113–123.

Hockings G.I., Grice J.E., Crosby G.V., Walters M.M., Jackson A.J. & Jackson R.V. (1993) Aspirin increases the human hypothalamic-pituitary-adrenal axis response to naloxone stimulation. *Journal of Clinical Endocrinology Metabolism* **77**, 404–408.

Ibeas C., Cejas J.R., Fores R., Badia P., Gomes T. & Hernandez A.L. (1997) Influence of eicosapentaenoic to docosahexanoic acid ratio (EPA/DHA) of dietary lipids on growth and fatty acid composition of gilthead seabream *Sparus aurata* juveniles. *Aquaculture* **150**, 91–102.

Jensen M.K., Madsen S.S. & Kristiansen K. (1998) Osmoregulation and salinity effects on the expression and activity of Na^+ K^+-ATPase in gills of European Sea Bass *Dicentrarchus labrax* L. *The Journal of Experimental Zoology* **282**, 290–300.

Koven W., Barr Y., Lutzky S., Ben-Atia I., Weiss R., Harel M., Behrens P. & Tandler A. (2001) The effect of dietary arachidonic acid (20:4n-6) on growth, survival and resistance to handling stress in gilthead seabream *Sparus aurata* larvae. *Aquaculture* **193**, 107–122.

Koven W.M. (2003) Key factors influencing juvenile quality in mariculture. *Israeli Journal of Aquaculture Bamdigeh* **55**, 283–297.

Koven W.M., Kissil G.W. & Tandler A. (1989) Lipid and n-3 requirement of *Sparus aurata* larvae during starvation and feeding. *Aquaculture* **79**, 185–192.

Lands W.E.M. (1991) Biosynthesis of prostaglandins. *Annual Review of Nutrition* **11**, 41–60.

Linares F. & Henderson R.J. (1991) Incorporation of ^{14}C-labelled polyunsaturated fatty acids by juvenile turbot, *Scophthalmus maximus* L. *in vivo*. *Journal of Fish Biology* **38**, 335–347.

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

McEvoy L., Estevez A., Bell J.G., Shields R.J., Gara B. & Sargent J.R. (1999) Influence of dietary levels of eicosapentanoic and arachidonic acids on the pigmentation success of turbot *Scophthalmus maximus* L. and halibut *Hippoglossus hippoglossus*. *Bulletin of the Aquaculture Association of Canada* **98**, 17–20.

Mourente G. & Tocher D.R. (1994) In vivo metabolism of [1-^{14}C] linolenic acid (18:3(n-3)) and [1-^{14}C] eicosapentaenoic acid (20:5(n-3)) in marine fish: time-course of the desaturation/elongation pathway. *Biochemica et Biophysica Acta* **1212**, 109–118.

Mourente G., Tocher D.R. & Sargent J.R. (1991) Specific accumulation of docosahexanoic acid (22:6n-3) in brain lipids during development of juvenile turbot *Scophthalmus maximus* L. *Lipids* **26**, 871–877.

Nye E.J., Hockings G.I., Grice J.E., Torpy D.J., Walters M.M., Crosbie G.V., Wagenaar M., Cooper M. & Jackson R.V. (1997) Aspirin inhibits vasopressin-induced hypothalamic-pituitary-adrenal activity in normal humans. *Journal of Clinical Endocrinology Metabolism* **82**, 812–817.

Rainuzzo J.R., Olsen Y. & Rosenlund G. (1989) The effect of enrichment diets on the fatty acids composition of the rotifer *Brachionus placatilis*. *Aquaculture* **79**, 157–161.

Rainuzzo J.R., Reiten 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) Origins and Functions of Egg Lipids. In: *Broodstock Management and Egg and Larval Quality* (ed. by N.R. Bromage & R.J. Roberts), pp. 353–372. Blackwell Science, Cambridge, MA, USA.

Sargent J.R., Bell G., McEvoy L., Tocher D. & Estevez A. (1999) Recent developments in the essential fatty acid nutrition of fish. *Aquaculture* **177**, 191–199.

Sorgeloos P., Dhert P. & Candreva P. (2001) Use of brine shrimp, Artemia spp., in marine fish larviculture. *Aquaculture* **200**, 147–159.

Tacon A.G. (1990) *Standard Methods for the Nutrition and Feeding of Farmed Fish and Shrimp*. Argent Laboratories Press, Redmond, WA, USA.

Takeuchi T., Toyota M., Satoh S. & Watanabe T. (1990) Requirement of juvenile red sea bream *Pagrus major* for eicosapentaenoic and docosahexeanoic acids. *Nippon Suisan Gakkaishi* **56**, 1263–1269.

Takeuchi T., Masuda R., Ishizaki Y., Watanabe T., Kanematsu M., Imaizumi K. & Tsukamoto K. (1996) Determination of the requirement of larval striped jack for eicosapentaenoic acid docosahexaenoic acid using enriched *Artemia* nauplii. *Fisheries Science* **62**, 760–765.

Van Anholt R.D., Koven W.M., Lutzky S. & Wendelaar Bonga S.E. (2004) Dietary supplementation with arachidonic acid alters the stress response of gilthead seabream (*Sparus aurata*) larvae. *Aquaculture* **238**, 369–383.

Watanabe T. (1982) Lipid nutrition in fish. *Comparative Biochemistry and Physiology* **73B**, 3–15.

Watanabe T. (1993) Importance of docosahexanoic acid in marine larval fish. *Journal of the World Aquaculture Society* **24**, 152–161.

Watanabe T., Kitajima C. & Fujita S. (1983) Nutritional values of live organisms used in Japan for mass propagation of fish: a review. *Aquaculture* **34**, 115–143.

Watanabe T., Izquierdo M.S., Takeuchi T., Satoh S. & Kitajima C. (1989) Comparison between eicosapentaenoic and docosahexaenoic acids in terms of essential fatty acid efficacy in larval red seabream. *Nippon Suissan Gakkaishi* **55**, 1635–1640.

Watanabe W.O., Smith T.J., Berlinsky D.L., Woolridge C.A., Stuart K.R., Copeland K.A. & Denson M.R. (2003) Volitional spawning of Black Sea Bass *Centropristis striata* induced with pelleted lutenizing hormone releasing hormone-analogue. *Journal of the World Aquaculture Society* **34**, 319–331.

Willey S., Bengtson D.A. & Harel M. (2003) Arachidonic acid requirements in larval summer flounder, *Paralichthys dentatus*. *Aquaculture International* **11**, 131–149.