

GROWTH, SURVIVAL AND RESISTANCE TO HYPERSALINE STRESS IN
LARVAL BLACK SEA BASS (*Centropristis striata*) FED VARYING LEVELS OF
DIETARY ARACHIDONIC ACID (20:4n-6)

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ABSTRACT

Significant advances have been made in controlled breeding and knowledge of environmental requirements for culture of larval black sea bass (Centropristis striata), but there still is relatively little published data on the nutritional requirements of the larval stages, including optimal methods for live prey enrichment with essential fatty acids. The objectives of this study were to determine the arachidonic acid (ARA, 20:4n-6) requirements of black sea bass larvae from the first feeding through metamorphic stages approximately 24 days post-hatching (d24ph).

Thirty 15-L aquaria were stocked with d1ph yolk sac stage larvae at 100 ind./L. Salinity (34 g/L), temperature (22 °C), photoperiod (18L: 6D), light intensity (1,000 lux), diffused aeration (100 mL/min) and D.O. (> 5 mg/L) were held constant. Background microalgae Nannochloropsis oculata was added daily to maintain 300,000 cells/L. To determine ARA requirements of larvae, live prey organisms, rotifers (Brachionus sp.) and Artemia were enriched with emulsions containing 10% docosahexaenoic acid, DHA (22:6n-3) and five different levels of ARA (0, 6, 8, 10 and 12% total fatty acids, TFA). In a sixth treatment, live prey was enriched with Algamac 2000 (26% DHA, 0% ARA), a commercial fatty acid booster. Rotifers were fed from d2ph at 10 ind./mL, increasing to 23 ind./mL by d18ph. On d18ph Artemia were fed at 0.5 ind./mL, increasing to 3 ind./mL on d22ph. Rotifer feeding ceased on d20ph. Larvae were sampled on d4, d10, d17 and d24ph to monitor survival and growth (NL, wet wt. and dry wt.). On d24ph, hypersaline (55 g/L) stress resistance (ST-50) was evaluated. To measure Na,K,ATP-ase expression, salinity was increased to a sublethal level of 42 g/L and larvae were sampled at 0 h and after 24 h for mRNA analysis by quantitative real-time PCR (qRT-PCR).

On d24ph, larval fatty acid profiles reflected dietary levels. Larval NL, wet wt. and dry wt. increased steadily in all treatments during the study, with no significant ($P > 0.05$) treatment effects. On d24ph, no significant treatment differences in survival (range = 24.3-32.7%) or hypersaline stress (range = 27.1-31.8) resistance were evident. However, larvae fed diets supplemented with ARA (6-12% TFA) demonstrated a significant ($P < 0.05$) increase in relative mRNA expression of $\text{Na}^+ \text{K}^+$ ATPase after 24 h, whereas larvae fed 0% ARA and Algamac showed no increase. The results indicate that dietary supplementation with ARA at 6-12% promoted the adaptive physiological responses to hypersalinity stress and hypo-osmoregulatory ability in black sea bass larvae.

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INTRODUCTION

Black sea bass: Life history and biology

The black sea bass, Centropristis striata, is a marine teleost in the family Serranidae and is currently being investigated as a candidate species for commercial aquaculture. Black sea bass can be found in shelf waters from Maine to Florida, and they spawn during the months of January through June after their first year of life (Mercer, 1989). Their larvae are pelagic and can be found in the inner continental shelf region. Juveniles are commonly seen in the vicinity of piers, jetties and wrecks, and adults are found in much deeper waters offshore (Mercer, 1989). Black sea bass are commercially fished with traps, hand lines, and trawls (Mercer, 1989). In 2004, the North Carolina Division of Marine Fisheries declared black sea bass over-fished in the area south of Cape Hatteras (NCDMF, 2005). This high demand placed on the commercial fishery to supply both local and niche markets has stimulated an interest in developing the techniques for the successful and reliable propagation of black sea bass through each of its life stages to a market size.

A bottleneck in the artificial propagation of sea bass is in reliable larviculture techniques to provide healthy fingerlings to grow-out operations. A number of studies have investigated the optimum environmental parameters necessary for successful production of black sea bass fingerlings, including temperature, photoperiod and salinity (Berlinsky et al., 2004). Relatively little experimental data are available on larval nutrition.

Larval Marine Finfish Nutrition

Marine finfish larvae typically have a very small yolk sac and are not capable of survival through metamorphosis on these endogenous reserves. After exhaustion of yolk reserves, marine finfish larvae, being carnivorous hunters, must be fed live prey before weaning to a microparticulate diet (Lavens et al., 1995, pp. 373-397). Marine fish larvae, such as black sea bass, typically have very small mouths and a commercially produced artificial pellet, manufactured in sizes small enough to contain all of the necessary nutrients and with the ability to remain intact in the water column, are currently not available. The nutritional requirements for marine fish larvae are species specific and can be determined by dose-response experiments (Sargent et al., 2002, pp. 182-219). The development of appropriate diets for first feeding larvae (<5 days post hatching) can be achieved by manipulating or enriching the available live feeds to contain prescribed levels of nutrients.

Sources of proteins used in fish feeds include soybean meal, krill meal, fish meal, and squid meal, and their quality is related to their amino acid composition (Kanazawa, 2003; Tacon, 1990). Larval protein requirements are similar to their body composition (Stickney, 2000). Sources of lipids used in fish feeds include plant and animal oils (Tacon, 1990). Some types of carbohydrates include cellulose, sucrose, fructose, glucose, lactose, starch and chitin (Tacon, 1990). They are not essential dietary components because they can be synthesized from proteins and lipids by a process called gluconeogenesis (Tacon, 1990).

Vitamins and minerals are required in trace amounts by fish and are not synthesized within the fish, therefore they must be provided in the diet (Tacon, 1990).

Sources of vitamins and minerals include yeasts, grains, plant and animal meals (Tacon, 1990). To date, very little is known about the vitamin and mineral requirements of marine fish larvae (Kanazawa, 2003; Tacon, 1990).

Lipid Nutrition in Larval Marine Finfish

Lipids are used in the development of the phospholipid bilayer which make up cell membranes and are precursors to a highly active group of local hormones called eicosanoids (Tacon, 1990; Bell et al., 1986). Lipids transport fat soluble vitamins A, D, E and K through the body and produce 9.5 kcal/g of energy (Watanabe, 1981; Tacon, 1990). Lipids commonly occur as triacylglycerols (TAG's) and are classified by their structure and polarity. Essential fatty acids (EFA's) are a fatty acid that the fish is unable to synthesize de novo (Tacon, 1990; Kanazawa, 2003). Known EFA's include docosahexaenoic acid (DHA, 22:6n-3), eicosapentaenoic acid (EPA, 20:5n-3) and arachidonic acid (ARA, 20:4n-6).

DHA is necessary for developing neural tissues, optical tissues and membrane structure (Mourente and Tocher, 1992; Furuita et al., 1998; Voet et al., 1999; Kanazawa, 2003; Valentine and Valentine, 2004). DHA is made from EPA which is made from linolenic acid (LN, 18:3n-3) through a process of desaturation and carbon chain elongation (Tacon, 1990 and Voet et al., 1999). ARA and EPA are precursors to a group of local hormones called eicosanoids such as prostaglandins, leukotrienes and thromboxanes (Voet et al., 1999; Bell and Sargent, 2003; Van Anholt et al., 2004). These local hormones are released during instances of stress and the inflammatory response

(Van Anholt et al., 2004). ARA is made from its precursor, linoleic acid (LL, 18:2n-6). Marine finfish are different from freshwater fish in that they are unable to synthesize DHA, EPA or ARA from their precursors LN or LL (Tacon, 1990; Kanazawa, 2003; Lavens et al., 1995, pp. 373-397). It has been determined that marine finfish lack the $\Delta 5$ desaturase and carbon chain elongation enzymes necessary to synthesize DHA, EPA or ARA from their precursor fatty acids, LL and LN (Kanazawa et al., 1979; Arts et al., 2001). It is also important to consider the competitive interactions between DHA and EPA or between EPA and ARA for the enzymes necessary to esterify them into phospholipids (Copeman et al., 2002). In turbot, Scophthalmus maximus, there is a deficiency in the C18-20 elongases, and the gilthead sea bream Sparus aurata lack the $\Delta 5$ desaturases necessary to convert polyunsaturated fatty acids into highly unsaturated fatty acids (Tocher and Ghioni, 1999).

Marine finfish must first be fed live feeds and then later weaned to a microparticulate diet prior to the metamorphic stage. The available live feeds, rotifers and Artemia, are low in the n-3 and n-6 fatty acids (Ackman, 1980). However, they can be enriched with fish, plant and fungus oils to contain various concentrations or ratios of EFA's (Harel et al., 2002). This enables the manipulation of the larval diets and the study of prescribed combinations of EFA's for the best growth, survival and resistance to stress.

Previous studies have demonstrated the importance of high levels of DHA compared to EPA in larval marine fish diets (Watanabe et al., 1989; Watanabe, 1993; Tocher and Sargent, 1984). DHA is incorporated in larval neural and optical tissues at a higher rate than EPA (Furuita et al., 1998; Mourente and Tocher, 1992; Valentine and

Valentine, 2004; Watanabe, 1989). Enrichment studies have shown that diets with high ratios of DHA to EPA (> 2) produce better stress resistance during hypersaline challenge tests in larval Japanese flounder Paralichthys olivaceus and red drum Scianops ocellatus (Lavens et al., 1995, pp. 373-397; Lee and Ostrowski, 2001). High ratios of DHA to EPA also improved survival in the larval Japanese flounder (Lavens et al., 1995, pp. 381). DHA has also been found to affect stress resistance in mahi mahi Coryphaena hippurus, Japanese flounder, striped mullet Mugil cephalus, milkfish Chanos chanos, and Asian sea bass Lates calcarifer (Kanazawa, 2003). High ratios of EPA to DHA caused an imbalance in the incorporation of these fatty acids into membrane structure of the turbot, ultimately leading to poor larval quality (Rainuzzo et al., 1997). In the red seabream Pagrus major, high ratios of EPA to DHA also led to poor larval quality (Watanabe, 1993).

Arachidonic Acid

In the roes of herring Clupea harengus, cod Gadus morhua, haddock Melanogrammus aeglefinus, whiting Merlangus merlangus, sand eel Amodytes lancea and capelin Mallotus villosus, the concentrations of n-3 to n-6 fatty acids averages 20:1 (Tocher and Sargent, 1984). Gilthead seabream larvae raised on the commercial diet Algamac 2000 (which is high in DHA (26%), but deficient in ARA) and two prepared diets, one with high DHA (35.9%) and no ARA and the other with high ARA to DHA (52 to 35.9%) showed better survival in the high ARA/DHA treatment (Koven et al., 2001).

In another study with gilthead seabream, increasing levels of ARA (0, 12.5, 25%) caused increasing cortisol levels in the hypothalamic pituitary interrenal (HPI) axis of larvae exposed to chronic hyperosmotic and hyposmotic stress (Koven et al., 2003). Larvae fed an intermediate level of ARA (12.5%) showed significantly higher survival when subjected to salinity changes than larvae fed the highest or lowest level of ARA (25%). Increasing levels of cortisol may enhance the sodium-potassium pumps in the gills, kidneys and intestines, which control the internal homeostasis of the larvae (Koven et al., 2003; Van Anholt et al., 2004).

Yellowtail flounder, Limanda ferruginea, larvae fed DHA/ARA (36.0/8.9%) exhibited lower growth and survival than those fed DHA alone (43.3%), but significantly higher growth than larvae fed DHA/EPA (37.4/14.2%), or the control with no DHA, EPA or ARA (Copeman et al., 2002). In turbot larvae, variations in dietary EPA/ARA produced no differences in growth or survival, but the medium and high ARA (7, 15%) treatments reduced pigmentation significantly (Estevez et al., 1999). Larval summer flounder Paralichthys dentatus fed increasing levels of dietary ARA (0, 3, 6, 9, 12% total lipid) showed highest survival, growth and stress resistance in the 6% ARA treatment (Willey et al., 2003). Larval striped trumpeter Latris lineata fed high levels of DHA and increasing levels of ARA, showed no differences in survival or growth compared to a commercial diet, Algamac 2000 (Bransden et al., 2004). Hypersaline stress tests administered on days 18 and 23 post-hatching, revealed that increasing levels of ARA lowered stress resistance, although no differences were observed on day 23. In black sea bass, larvae fed diets containing 10% DHA showed better growth and survival than those fed 0% DHA. Increasing ARA within the range of 0-6% improved growth and survival

from first feeding through metamorphic stages (Rezek et al., 2005). These findings point to the importance of diets that contain high levels of DHA compared to EPA or ARA. A better understanding of the role and necessity of ARA in larval nutrition is needed.

Objectives

The objectives of this study were to evaluate the effects of varying levels of dietary arachidonic acid (20:4n-6) on growth, survival, resistance to stress and osmoregulation in larval black sea bass (Centropristis striata). To determine the role ARA plays in the osmoregulatory response of black sea bass larvae, the relative mRNA expression of Na⁺ K⁺ ATPase was measured. Larval performance was compared with those fed a popular commercial diet deficient in ARA. Based on these results, we hope to recommend an optimum dietary level of ARA to be applied in commercial larviculture.

The Null Hypothesis to be tested

There will be no differences in growth, survival, stress resistance or relative mRNA expression of Na, K, ATPase among black sea bass larvae fed live prey organisms enriched to contain different levels of dietary ARA (0, 6, 8, 10, and 12%).

METHODS

Experimental Animals

This experiment was conducted at the University of North Carolina Wilmington Center for Marine Science Aquaculture Facility, Wrightsville Beach, NC. Wild-caught black sea bass broodstock were maintained in recirculating aquaculture tanks located on site. Brood tanks had a working volume of 2,134-L and were provided with heat pumps and lights to control photoperiod and temperature. Seawater of 34 ppt was pumped from the adjacent Atlantic intracoastal waterway and was filtered (1- μm) and UV-sterilized. Spawning was induced using a Luteinizing Hormone Releasing Hormone-analogue pellet implanted in a sexually mature female in the post-vitellogenic stage of oocyte development (Berlinsky et al., 2000; Watanabe et al., 2003; Berlinsky et al., 2004). Eggs were collected from brood tanks and viable (buoyant) eggs were separated from nonviable (non-buoyant) eggs. Fertilized, buoyant eggs were incubated in a 125-L tank in 34 ppt seawater and at 18 C until hatching. Newly hatched (approx 52 h post-fertilization) yolk-sac larvae were randomly distributed from the incubator into experimental aquaria.

Experimental System

The experimental system consisted of four temperature-controlled water baths situated in an indoor laboratory. Water in each bath was circulated through a heater/chiller and temperature was regulated by a digital thermostat. The experimental units were 15-L, cylindrical, black tanks. Each water bath contained 8 tanks and was enclosed by an opaque black plastic curtain with an overhanging light hood to provide

artificial lighting. The light hood contained 40-W daylight fluorescent light bulbs controlled by digital timers for photoperiod manipulation and were adjusted vertically for surface light intensity.

Experimental Design

To determine the effects of dietary ARA, black sea bass larvae were fed live prey organisms, rotifers and Artemia, enriched to contain 10% DHA and five different levels of ARA: 0, 6, 8, 10 and 12% total fatty acids (TFA). In addition, one group of larvae were fed live prey organisms enriched with a popular commercial fatty acid booster for marine finfish larvae (Algamac 2000, Aquafauna Bio-Marine, Hawthorne, CA, USA), containing 26% DHA, 0.6% EPA and no ARA. Larvae were reared through metamorphosis, around day 24 post hatching.

Experimental Conditions

To begin the experiment, larvae were stocked in each tank at a density of 100 ind./L. Environmental conditions, including salinity (35 ppt), temperature (22 °C), photoperiod (18L: 6D), light intensity (1,000 lux), diffused aeration (100 ml/min) and dissolved oxygen (> 5 mg/L) were held constant throughout the experiment. Dissolved oxygen, pH, salinity, lux, and aeration levels were measured daily in each replicate tank. Background micro-algae Nannochloropsis oculata (Reed Mariculture, Campbell, CA, USA) was added daily to maintain a density of 300,000 cells/L to promote water quality and to provide a source of vitamins and minerals. To maintain water quality, each tank was stirred and siphoned using a screen of appropriate mesh size to remove 5 L, or 1/3 of

the tank volume daily. Filtered and sterilized seawater was then added to each tank and surface oils were removed using a paper towel.

Feeds

Rotifers were grown at the UNCW aquaculture facility on a non-viable microalgae Nannochloropsis oculata (Reed Mariculture, Campbell, CA, USA). Rotifers Brachionus plicatilis were enriched with the different treatment emulsions for 8 h before feeding to the fish larvae. Artemia nauplii hatched from decapsulated cysts were enriched with the different treatment emulsions for 16 h before feeding to the larvae. Prey fatty acid profiles were confirmed by gas chromatography.

Rotifers were introduced to the rearing tanks on day 2 post-hatching at a rate of 10/ml until first feeding on day 3 post hatching, at which time the density was increased to 15/ml and then to a maximum of 23/ml by day 18 post-hatching. Artemia nauplii were added in addition to rotifers on day 18 post-hatching at a density of 0.5/ml. On day 20 post-hatching, the Artemia nauplii density was increased to 2/ml and rotifer feeding ceased. On day 22 post-hatching, Artemia nauplii density was increased to 3.0/ml for the remainder of the experiment.

Live Prey Enrichment

The DHA/ARA emulsions were formulated by using different proportions of DHAasco and ARAsco. DHAasco is a DHA-rich single cell oil and is extracted from the heterotrophically grown algae Cryptocodinium cohnii. ARAsco is an ARA-rich single cell oil and it is extracted from the fungus Mortirella alpina. These triglyceride oils were

mixed with olive oil and water with 5% lecithin, 1% ascorbic acid and 1% Tween-80 (with oil weight) (Advanced Bionutrition, Columbia, MD, USA). For enrichment of rotifers, 0.1 g/0.5x10⁶ every four hours and were harvested after 8 h. Artemia, were enriched with 0.3g/0.25x10⁶ every eight hours and were harvested after 16 h.

Sampling and Data Collection

Using volumetric methods, sampling was conducted at the start of the study on d4, d10, d17 and d24ph. A target sample size of ten larvae were collected. The volume necessary to collect at least ten larvae was then used to determine the density of larvae at each sample period. At each sampling, growth was determined in anesthetized (0.3-0.5 ppt phenoxyethanol) larvae from notochord length using a microscope fitted with an ocular micrometer. Larval wet and dry weights were measured to the nearest 10 µg using a Sartorius (Sartorius AG, Goettingen, Germany) electronic balance. Survival was determined from larval densities at each sampling as a percentage of initial density.

Carcass Analysis

Approximately 200 mg (wet weight) of black sea bass larvae were taken from replicate tanks on d24ph. Excess water was removed and larvae were stored (- 20° C) under nitrogen gas in cryogenic vials (Fisher scientific, USA) until lipid extraction. Using a revised Folch et al. (1957) method, lipids were extracted from whole larval tissues in 1:1 chloroform/methanol by homogenization and sonication. Samples were then filtered and solvent was removed using a rotary evaporator (Buchi labortechnik AG, Meierseggstrasse, Switzerland). The extract was then dissolved in 1:1

chloroform/methanol, filtered and transferred into a pre-weighed 50 ml round bottom flask. The solvent was roto-evaporated to dryness and the remaining lipid was weighed to determine percent total lipid.

Lipids were converted into fatty acid methyl esters (FAME's), first by dissolving in 900 μ L of 0.5 M NaOH/MeOH and heating (between 70 and 100° C) for 30 min. This reaction breaks the glycerol backbone of the triacylglycerols. After the 30 min reaction, 1.5 mL of boron trifluoride-methanol (BF₃) was added, followed by another reaction period of 30 min at 70 - 100° C. The reaction with BF₃/Methanol esterifies the free fatty acids to give their methyl esters (FAME's).

Isolation of FAME's at room temperature (25° C) was accomplished by the addition of 1 mL of saturated NaCl and 1 mL of 100% hexane. Using a Pasteur pipette, the organic layer was separated from the aqueous layer and filtered through 32 μ m silica column. The column was rinsed with 1 mL of 50% ether/hexane two times to remove the entire sample. FAME's were roto-evaporated and suspended in 400 μ L of 100% chloroform for GC analysis.

FAME's were identified using a Hewlett Packard 6890 gas chromatography flame-ionization-detector (GC-FID) (Hewlett Packard, USA). Using helium as the carrier gas, injected samples passed through the column in approximately 15 min. The column was initially heated to 195° C and held for 8 min, ramped at a rate of 15° C/min and held at 270° C for the remaining 2 min. FAME peaks were identified using the known standards GLC-84, docosapentaenoic acid methyl ester n-3, docosapentaenoic acid methyl ester n-6 (Nu Check Prep, Elysian, MN, USA), ecosapentaenoic acid methyl

ester, arachidonic acid methyl ester (Sigma Aldrich, St Louis, MO, USA) and stearidonic acid methyl ester (Cayman Chemical, USA).

mRNA Expression of Na⁺ K⁺ ATPase

On d25ph black sea bass larvae were subjected to a less-than-lethal hyperosmotic salinity challenge of 43 ppt. In killifish Fundulus heteroclitus and European sea bass Dicentrarcus labrax, larvae subjected to an increase in salinity had a significant increase in expression levels of Na⁺ K⁺ ATPase after 24 hours of exposure (Jensen et al., 1998; Scott et al., 2004). In this study, we chose to sample larvae before increasing the salinity (0 h) and after 24 h. Due to the small size of the larvae, ten larvae were pooled for each sample and stored at -20° C in RNAlater (Ambion, Woodward, Texas, USA) to preserve the integrity of RNA until it could be extracted from larval tissues.

RNA Extraction and Reverse Transcription

Total RNA was extracted using an RNeasy Mini Kit (Qiagen, Valencia, California, USA). The protocol for animal tissues was modified by using Trizol (Invitrogen, Carlsbad, California, USA) instead of Buffer RLT (Qiagen, Valencia, California, USA). RNA was bound to a column with 70 % ethanol and then eluted with 30 µl of nuclease-free water. Maximum RNA yield was obtained by eluting a second time with the 30 µl eluant. Concentrations of RNA samples were assessed using an Agilent 2100 Bioanalyzer (Agilent, Palo Alto, California). To normalize every sample, 4 µg of total RNA was used for first strand cDNA synthesis.

Sequencing of Na⁺ K⁺ ATPase

Gill tissue excised from juvenile fish was used to obtain partial sequence of the black sea bass Na⁺ K⁺ ATPase (NKA). The same protocols for RNA extraction and reverse transcription were followed. Degenerate primers (Forward: 5'-ATG CAN GTN GCN CAY ATG TGG-3' and Reverse: 5'-GGN GGR TCD ATC ATN GCC AT-3') were designed from known NKA sequences obtained through GenBank (Pubmed) and aligned using ClustalW (EMBL-EBI, European Bioinformatics Institute). Restriction fragment length polymorphisms produced through traditional PCR, using degenerate primers, were verified with electrophoresis and purified using a Wizard SV Gel & Cleanup Kit (Promega, Madison, Wisconsin, USA). A sequencing reaction was used with this product in the presence of Big Dye Terminator Ready Reaction Mix (Applied Biosystems, Foster City, California, USA). A sephadex column was used to clean up the sequencing reaction product. The eluant was then speed-vac'd to dryness and resuspended in 10 µl of Hi-Dye (Applied biosystems, Foster City, California, USA) then transferred to a sequencing plate. Forward and reverse sequences were obtained from a capillary DNA sequencer, ABI 3100 (Applied Biosystems, Foster City, California, USA). They were checked and assembled using Vector NTI (Invitrogen, Carlsbad, California, USA) to obtain partial sequence of NKA. This segment of the NKA gene was verified using BLAST (Pubmed) and the partial sequence was stored in Genbank (accession number: DQ492680).

Quantitative PCR

Quantitative real-time PCR was accomplished using qPCR primers (Forward: 5'-AGC CTA TGG ACG ACG AGA TGA-3' and Reverse: 5'-CCA GCT CAA CAT AGG CGT TCT-3') designed in Primer Express (Applied Biosystems, Foster City, California, USA) from the partial sequence of the black sea bass NKA (genbank accession number: DQ492680). All samples were analyzed in the presence of SYBR green fluorescent Dye (Applied Biosystems, Foster City, California, USA) using an Agilent 7500 quantitative PCR system (Agilent, Palo Alto, California, USA). To quantify the relative expression of NKA between samples the same standard was used for both sample periods (0, 24 h). The $\text{Na}^+ \text{K}^+$ ATPase gene expressed in larvae fed 8% ARA and sampled 24 h after salinity transfer was used as a standard for expression analysis at both time periods. This standard consisted of a full-strength (1.0) and a dilution series of 0.5, 0.25, and 0.125. The standard was used to show differences in linearity between the log concentration and the critical threshold cycle number of each sample. Transcript abundance for all samples were reported relative to the standard.

Hypersaline Stress Resistance

A hypersaline stress test was administered to a sample of larvae from each replicate tank on d24ph. A stress sensitivity index (ST50), the median survival time (MST) index, was used (Dhert et al., 1990). This is the time at which larval survival falls to 50% following exposure of a group of 20 larvae to a hypersaline challenge of approximately 55 ppt salinity seawater. In addition, mean survival time was calculated to evaluate stress resistance (Watanabe et al., 1985).

Data Analysis

All data were expressed as mean \pm standard error of the mean (SEM). After homogeneity of variances was determined, treatment means were compared by a one-way ANOVA. A Welch ANOVA was used for unequal standard deviations. Multiple comparisons among means were performed using Tukey's test. Survival data were arcsine transformed for statistical analysis (Sokal and Rohlf, 1989). A significance level of $P \leq 0.05$ was used in all tests. Statistical software JMP IN version 4 (SAS Institute Inc., Cary, North Carolina, USA) was used to perform computational analyses.

RESULTS

Fatty Acid Composition of Enriched Rotifers

The fatty acid composition (% Total Fatty Acids or TFA's) of enriched rotifers is reported in Table 1. No significant ($P < 0.05$) treatment effects were observed in total lipid ($\bar{X} = 9.74$). Saturated fatty acids (SFA's) showed significant ($P < 0.05$) treatment differences. Rotifers enriched with Algamac 2000 (ALG) had higher ($P < 0.05$) total SFA ($\bar{X} = 30.3$) than 0, 6 and 8% ARA treatments ($\bar{X} = 22.2 - 22.9$). Myristic acid (14:0), palmitic acid (16:0) and stearic acid (18:0) showed significant ($P < 0.05$) treatment differences. Rotifers enriched with ALG had higher ($P < 0.05$) levels of 14:0 ($\bar{X} = 5.66$) than the ARA treatments ($\bar{X} = 3.67 - 4.54$). Concentrations of 16:0 were higher ($P < 0.05$) in the ALG treatment ($\bar{X} = 21.7$) than in the 0, 6, 8 and 12% ($\bar{X} = 15.5 - 16.7$) ARA treatments. Levels of 18:0 were higher ($P < 0.05$) in the 10% ARA treatment ($\bar{X} = 4.28$) than in the 0, 6% ARA and ALG treatments ($\bar{X} = 2.13 - 2.93$).

A significant ($P < 0.05$) trend toward decreasing monounsaturated fatty acids (MUFA's) with increasing ARA was observed from 43.0 in 0% ARA to 25.4 in 12% ARA (Fig. 1). Of the MUFA's detected, no treatment ($P > 0.05$) effects were observed in 20:1 (eicosenoic acid) ($\bar{X} = 1.69$) or 16:1n-7 (palmitoleic acid) ($\bar{X} = 8.8$) (Table 1). A significant ($P < 0.05$) trend toward lower 18:1n-9 (oleic acid) with increasing ARA was observed from 32.5% at 0% ARA to 15.4 at 12% ARA and 5.53 in ALG (Fig. 2).

Significant ($P < 0.05$) treatment effects were observed in the concentrations (% TFA's) of n-3 polyunsaturated fatty acids (PUFA's) (Fig. 3). The concentration of n-3 PUFA's was higher ($P < 0.05$) in the ALG treatment ($\bar{X} = 37.1$) than in all other

treatments ($\bar{X} = 24.4$). Of the n-3 PUFA's detected, there were no significant ($P > 0.05$) treatment effects observed for 20:5n-3 (eicosapentanoic acid) (Table 1). Significant ($P < 0.05$) treatment effects were observed for 18:3n-3 (linolenic acid), 18:4n-3 (stearidonic acid), 22:5n-3 (docosapentaenoic acid) and 22:6n-3 (docosahexaenoic acid). The concentration of 18:3n-3 was higher ($P < 0.05$) in ALG ($\bar{X} = 4.26$) than in the ARA treatments ($\bar{X} = 2.51 - 3.12$). Of the ARA treatments, the 0% ($\bar{X} = 3.12$) ARA contained higher ($P < 0.05$) levels of 18:3n-3 than the 6, 8, 10 and 12% ARA treatments ($\bar{X} = 2.28 - 2.67$) (Fig. 4). Concentrations of 18:4n-3 were higher ($P < 0.05$) in the 12% ($\bar{X} = 1.29$) ARA treatment than in the ALG treatment ($\bar{X} = 0.45$) (Fig.). A significant ($P < 0.05$) trend of increasing 18:4n-3 with increasing ARA levels was detected from 0% at 0% ARA to 1.29 at 12% ARA (Fig. 5). The concentration of 22:5n-3 was higher ($P < 0.05$) in the ALG ($\bar{X} = 3.71$) and 10% ARA treatments ($\bar{X} = 3.36$) than the 0, 6, 8 and 12% ARA ($\bar{X} = 2.90 - 3.19$) treatments (Table 1). The concentration of 22:6n-3 was higher ($P < 0.05$) in the ALG treatment ($\bar{X} = 19.8$) than the ARA treatments ($\bar{X} = 10.1$) (Fig. 6). Significant ($P < 0.05$) treatment effects were observed on the ratio of 22:6n-3/20:5n-3. The ALG treatment had a significantly higher ($P < 0.05$) ratio of 22:6n-3/20:5n-3 ($\bar{X} = 2.29$) than the 0 and 6% ARA treatments ($\bar{X} = 1.23 - 1.27$) (Fig. 7), but not the 8, 10 and 12% ARA treatments ($\bar{X} = 1.32 - 1.50$).

A significant ($P < 0.05$) trend toward increasing levels of n-6 PUFA's with increasing levels of ARA was observed from 10.3 at 0% ARA to 21.9 at 12% ARA (Fig. 8). The ALG treatment ($\bar{X} = 12.2$) had significantly ($P < 0.05$) less n-6 PUFA than the 6, 8, 10 and 12% ARA treatments ($\bar{X} = 16.5 - 20.1$) but was not significantly ($P > 0.05$) different from the 0% ARA treatment ($\bar{X} = 10.3$). Of the n-6 PUFA's detected,

significant ($P < 0.05$) differences were observed for 18:2n-6 (linoleic acid) and 20:4n-6 (arachidonic acid). The concentration of 18:2n-6 was lower ($P < 0.05$) in ALG ($\bar{X} = 3.28$) than in ARA treatments ($\bar{X} = 7.66 - 8.71$) (Fig. 9). A significant ($P < 0.05$) trend toward increasing 20:4n-6 with increasing dietary ARA was observed from 1.62 at 0% ARA to 14.3 at 12% ARA (Fig. 10). Levels of 20:4n-6 in the ALG treatment ($\bar{X} = 2.88$) did not differ ($P > 0.05$) from 0% ARA, but were lower than the 6 – 12% ARA treatments. Docosapentaenoic acid (22:5n-6), was found in rotifers enriched with ALG ($\bar{X} = 6.05$) but was not detected in any of the ARA treatments (Table 1).

The ratio of n-3/n-6 PUFA was significantly ($P < 0.05$) higher in the ALG ($\bar{X} = 3.03$) than ARA treatments (Fig. 11). Among the ARA treatments, a trend ($P < 0.05$) toward lower n-3/n-6 PUFA's was observed from 2.31 at 0% ARA to 1.12 at 12 ARA. Both ALG ($\bar{X} = 6.86$) and 0% ARA ($\bar{X} = 6.16$) had higher ($P < 0.05$) levels of 22:6n-3/20:4n-6 than those detected in the 6, 8, 10 and 12% ARA treatments ($\bar{X} = 0.71 - 1.30$) (Fig. 13). The ratio of 20:5n-3/20:4n-6 was higher ($P < 0.05$) in the 0% ARA ($\bar{X} = 4.83$) than in the 6 – 12% ARA treatments ($\bar{X} = 0.54 - 1.11$) (Fig. 12). ALG had a higher ($P < 0.05$) ratio of 20:5n-3/20:4n-6 ($\bar{X} = 3.07$) than the 6-12% ARA treatments.

Table 1. Fatty acid composition (% of total FAMES) of enriched rotifers *Brachionus plicatilis*. Values represent means \pm SEM, (N=3).

Enriched diets received by larvae (% total lipid as ARA and Algamac 2000)						
Fatty acids	0%	6%	8%	10%	12%	Algamac 2000
14:0	3.76 \pm 0.05 a	4.54 \pm 0.19 a	3.67 \pm 0.024 a	4.31 \pm 0.17 a	3.87 \pm 0.20 a	5.66 \pm 0.20 b
16:0	15.5 \pm 0.71 a	15.5 \pm 0.76 a	15.8 \pm 1.37 a	17.4 \pm 0.99 ab	16.7 \pm 0.25 a	21.7 \pm 1.26 b
16:1n-7	8.46 \pm 0.33	10.0 \pm 0.84	8.14 \pm 0.70	7.60 \pm 0.59	8.39 \pm 0.24	10.2 \pm 0.59
18:0	2.86 \pm 0.14 ab	2.13 \pm 0.03 b	3.38 \pm 0.23 ad	4.28 \pm 0.45 d	4.02 \pm 0.30 ad	2.93 \pm 0.17 ab
18:1n-9	32.5 \pm 0.74 a	23.1 \pm 1.88 b	20.9 \pm 0.25 bc	18.4 \pm 0.50 cd	15.4 \pm 0.28 d	5.53 \pm 0.32 e
18:2n-6	8.71 \pm 0.69 a	8.24 \pm 0.21 a	7.79 \pm 0.18 a	8.43 \pm 0.31 a	7.66 \pm 0.38 a	3.28 \pm 0.24 b
18:3n-3	3.12 \pm 0.06 a	2.56 \pm 0.05 bc	2.67 \pm 0.08 b	2.28 \pm 0.03 c	2.51 \pm 0.08 bc	4.26 \pm 0.11 d
18:4n-3	0	0.53 \pm 0.27 ab	0.90 \pm 0.02 ab	1.21 \pm 0.05 ab	1.29 \pm 0.10 b	0.45 \pm 0.23 a
20:1	1.95 \pm 0.06	1.26 \pm 0.22	1.77 \pm 0.27	1.74 \pm 0.14	1.63 \pm 0.26	1.81 \pm 0.11
20:4n-6	1.62 \pm 0.21 a	8.27 \pm 0.27 b	9.54 \pm 0.09 b	11.6 \pm 0.64 c	14.3 \pm 0.26 d	2.88 \pm 0.04 a
20:5n-3	7.74 \pm 0.64	9.12 \pm 1.00	7.37 \pm 0.63	6.83 \pm 0.33	7.75 \pm 0.51	8.83 \pm 0.59
22:5n-3	3.19 \pm 0.09 bc	2.97 \pm 0.07 bc	2.90 \pm 0.07 c	3.36 \pm 0.14 ab	2.91 \pm 0.09 c	3.71 \pm 0.09 a
22:5n-6	0	0	0	0	0	6.05 \pm 0.69
22:6n-3	9.72 \pm 0.62 a	10.8 \pm 0.54 a	9.95 \pm 0.34 a	10.2 \pm 0.51 a	10.1 \pm 0.12 a	19.8 \pm 2.04 b
Σ Saturates	22.2 \pm 0.78 a	22.2 \pm 0.90 a	22.9 \pm 1.82 a	26.0 \pm 1.51 ab	24.6 \pm 0.51 ab	30.3 \pm 1.44 b
Σ Monounsaturates	43.0 \pm 0.74 a	34.3 \pm 0.85 b	30.8 \pm 1.08 c	27.7 \pm 0.60 cd	25.4 \pm 0.29 de	23.6 \pm 0.32 e
Σ n-3 Polyunsaturates	23.8 \pm 1.03 a	25.9 \pm 0.52 a	23.8 \pm 0.45 a	23.9 \pm 0.63 a	24.6 \pm 0.52 a	37.1 \pm 1.82 b
Σ n-6 Polyunsaturates	10.3 \pm 0.61 a	16.5 \pm 0.48 b	17.3 \pm 0.14 bc	20.1 \pm 0.93 cd	21.9 \pm 0.60 d	12.2 \pm 0.52 a
n-3/n-6 Polyunsaturates	2.31 \pm 0.14 a	1.58 \pm 0.08 b	1.37 \pm 0.03 bc	1.19 \pm 0.04 c	1.12 \pm 0.05 c	3.03 \pm 0.07 d
DHA/EPA	1.27 \pm 0.12 a	1.23 \pm 0.21 a	1.38 \pm 0.17 ab	1.50 \pm 0.12 ab	1.32 \pm 0.11 ab	2.29 \pm 0.38 b
DHA/ARA	6.16 \pm .070 a	1.30 \pm 0.03 b	1.04 \pm 0.03 b	0.88 \pm 0.01 b	0.71 \pm 0.01 b	6.86 \pm 0.64 a
EPA/ARA	4.83 \pm 0.22 a	1.11 \pm 0.16 c	0.77 \pm 0.07 c	0.59 \pm 0.05 c	0.54 \pm 0.04 c	3.07 \pm 0.24 b
Total Lipid	9.63 \pm 0.84	9.27 \pm 0.36	10.1 \pm 0.48	9.93 \pm 0.85	10.1 \pm 0.14	9.43 \pm 0.79

(% Wet tissue Weight)

Mean values within rows that are not followed by the same letters are significantly ($P < 0.05$) different.

Monounsaturated fatty acids (MUFA's)

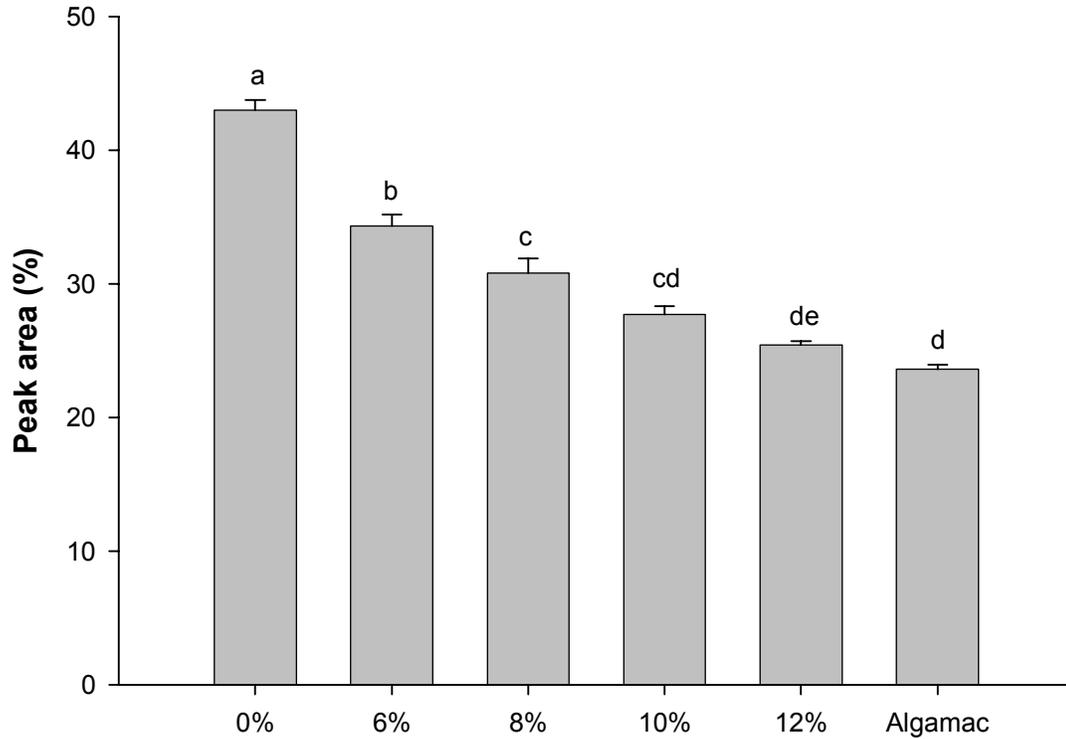


Figure 1. Percentage of monounsaturated fatty acids, MUFA's ($\bar{X} \pm \text{SEM}$, $N = 3$) detected in rotifers enriched with five different oil emulsions and a commercial diet, Algamac 2000. Each oil emulsion contained 10% DHA and different levels of ARA (0, 6, 8, 10, 12% total lipid). Treatment means not associated with the same letters are significantly different ($P < 0.05$).

18:1n-9 (Oleic acid)

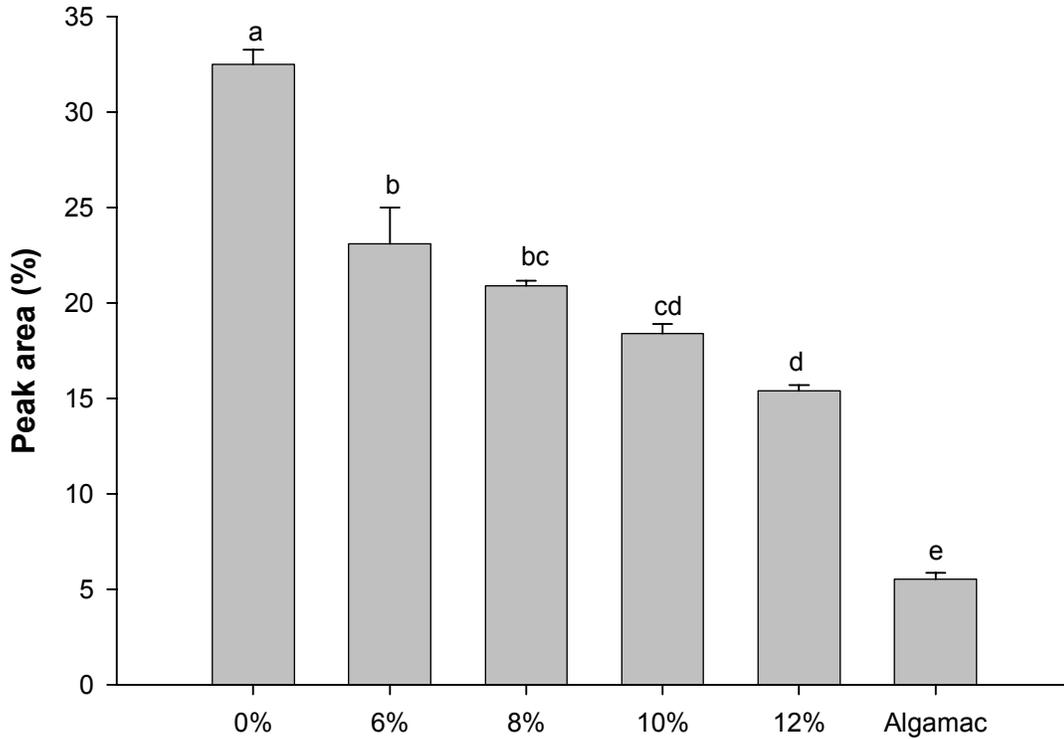


Figure 2. Percentage of oleic acid, 18:1n-9 ($\bar{X} \pm \text{SEM}$, $N = 3$) detected in rotifers enriched with five different oil emulsions and a commercial diet, Algamac 2000. Each oil emulsion contained 10% DHA and different levels of ARA (0, 6, 8, 10, 12% total lipid). Treatment means not associated with the same letters are significantly different ($P < 0.05$).

n-3 Polyunsaturated fatty acids (PUFA's)

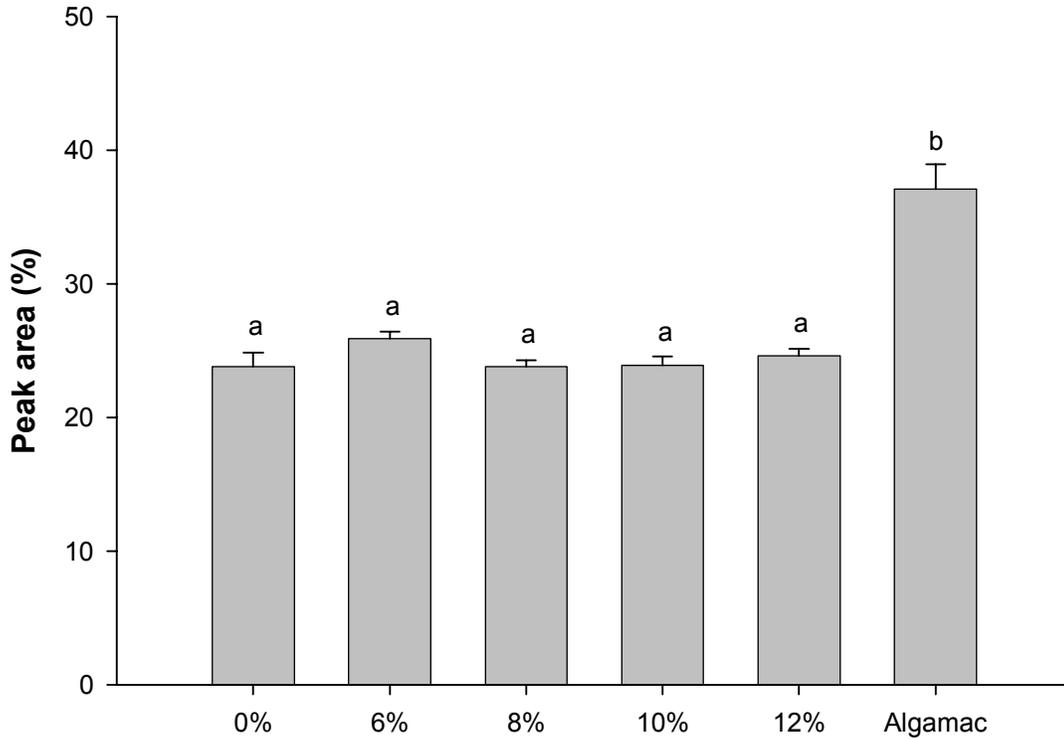


Figure 3. Percentage of n-3 polyunsaturated fatty acids (PUFA's) ($\bar{X} \pm \text{SEM}$, $N = 3$) detected in rotifers enriched with five different oil emulsions and a commercial diet, Algamac 2000. Each oil emulsion contained 10% DHA and different levels of ARA (0, 6, 8, 10, 12% total lipid). Treatment means not associated with the same letters are significantly different ($P < 0.05$).

18:3n-3 (Linolenic acid)

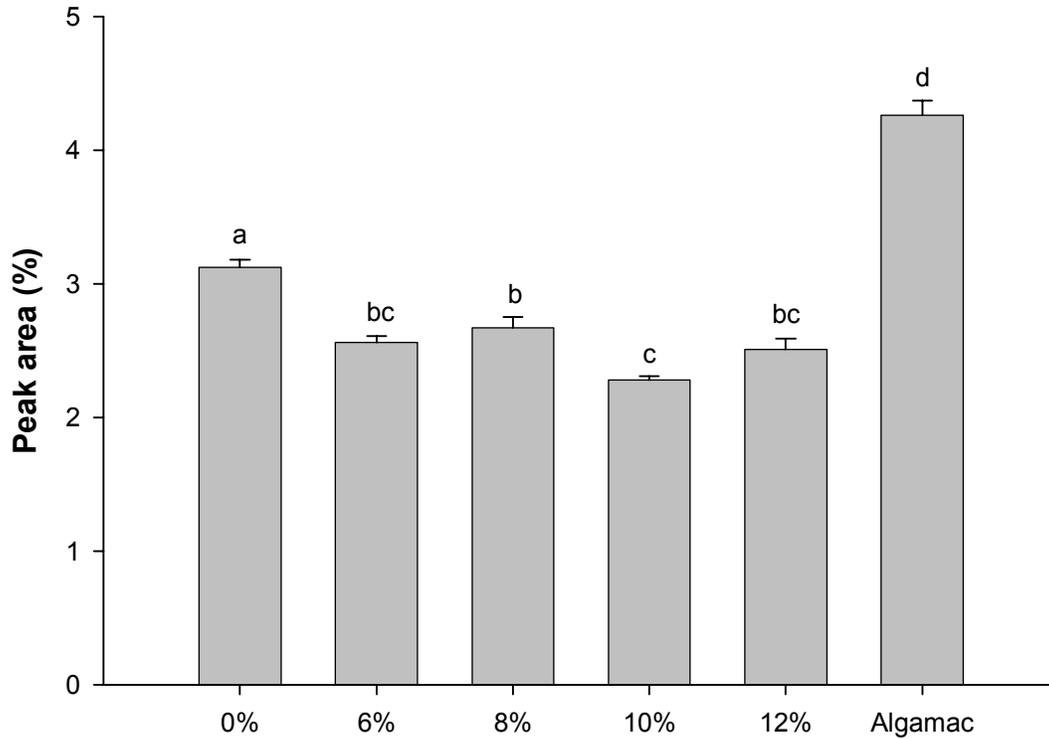


Figure 4. Percentage of linolenic acid, 18:3n-3 ($\bar{X} \pm \text{SEM}$, $N = 3$) detected in rotifers enriched with five different oil emulsions and a commercial diet, Algamac 2000. Each oil emulsion contained 10% DHA and different levels of ARA (0, 6, 8, 10, 12% total lipid). Treatment means not associated with the same letters are significantly different ($P < 0.05$).

18:4n-3 (Stearidonic acid)

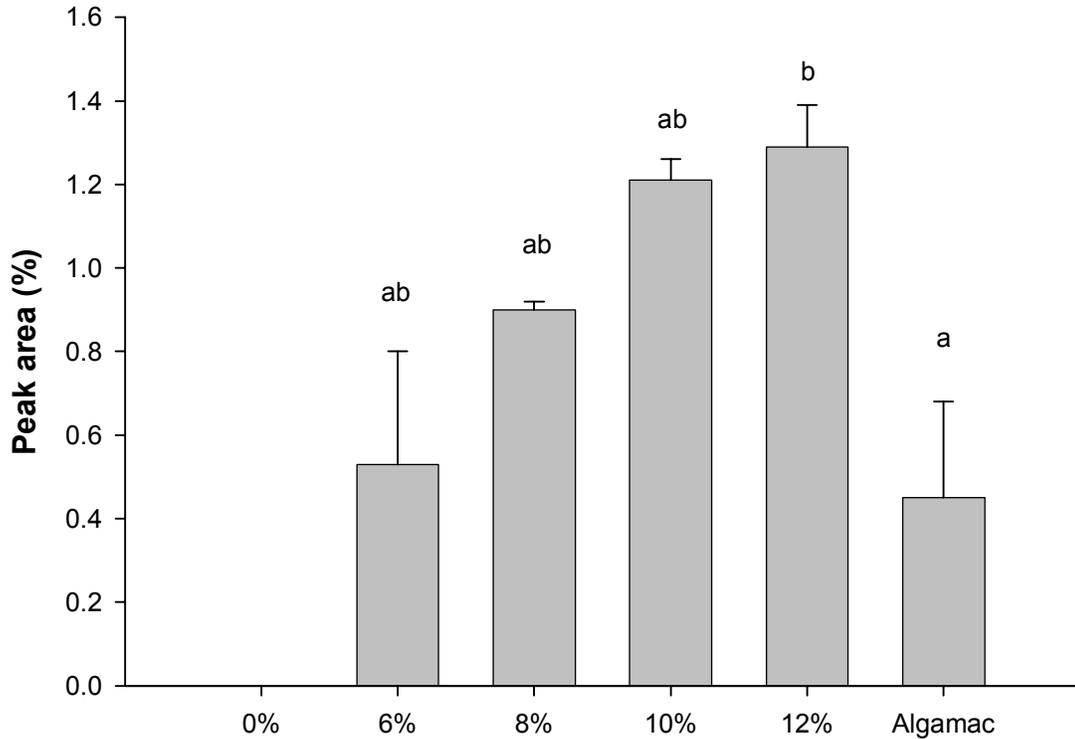


Figure 5. Percentage of stearidonic acid, 18:4n-3 ($\bar{X} \pm \text{SEM}$, $N = 3$) detected in rotifers enriched with five different oil emulsions and a commercial diet, Algamac 2000. Each oil emulsion contained 10% DHA and different levels of ARA (0, 6, 8, 10, 12% total lipid). Treatment means not associated with the same letters are significantly different ($P < 0.05$). There was no stearidonic acid detected in the 0% ARA treatment.

22:6n-3 (Docosahexaenoic acid)

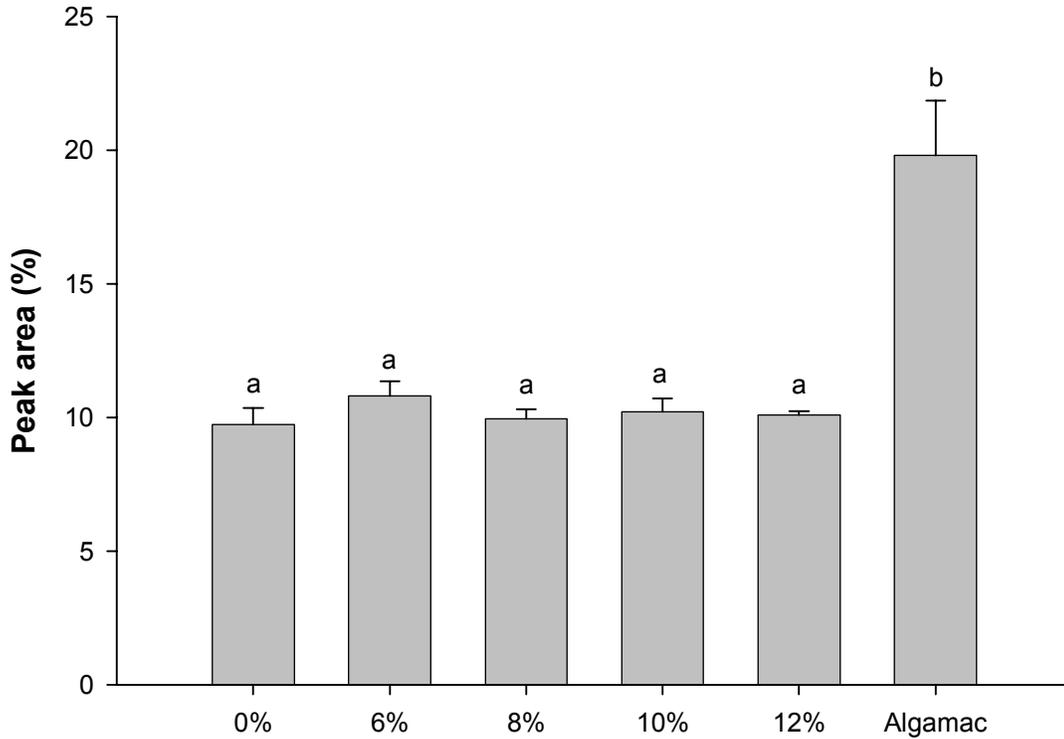


Figure 6. Percentage of docosahexaenoic acid, 22:6n-3 ($\bar{X} \pm \text{SEM}$, $N = 3$) detected in rotifers enriched with five different oil emulsions and a commercial diet, Algamac 2000. Each oil emulsion contained 10% DHA and different levels of ARA (0, 6, 8, 10, 12% total lipid). Treatment means not associated with the same letters are significantly different ($P < 0.05$).

22:6n-3/20:5n-3

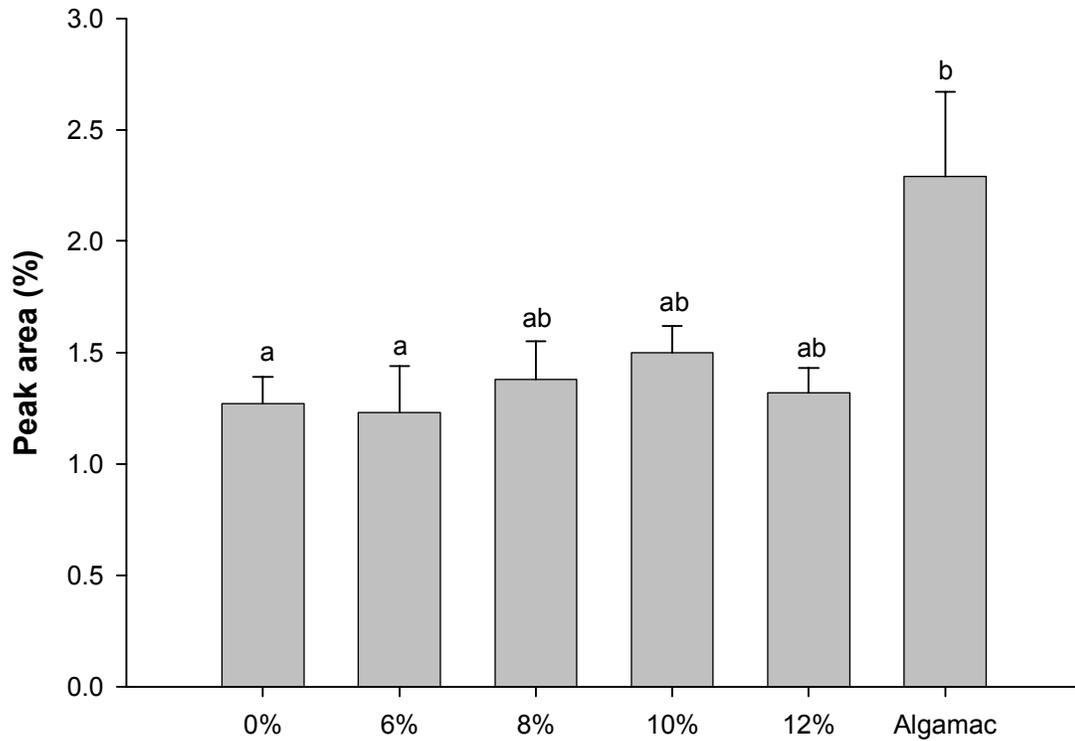


Figure 7. Ratio of 22:6n-3/ 20:5n-3 ($\bar{X} \pm \text{SEM}$, $N = 3$) detected in rotifers enriched with five different oil emulsions and a commercial diet, Algamac 2000. Each oil emulsion contained 10% DHA and different levels of ARA (0, 6, 8, 10, 12% total lipid). Treatment means not associated with the same letters are significantly different ($P < 0.05$).

n-6 Polyunsaturated fatty acid (PUFA)

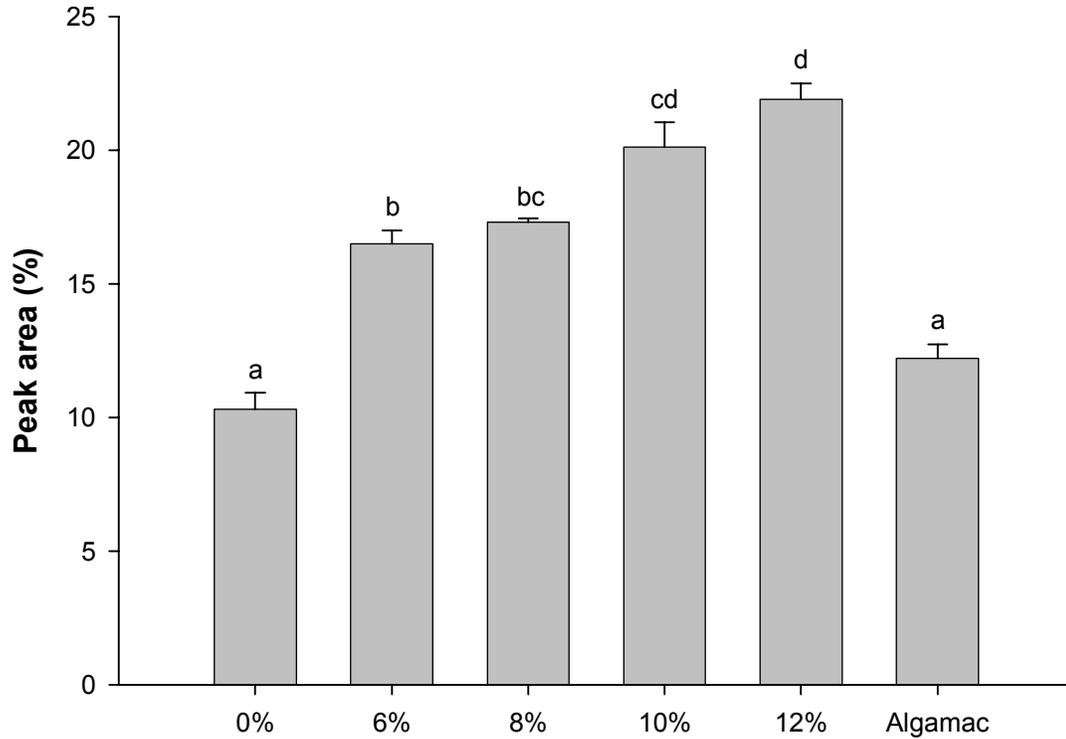


Figure 8. Percentage of n-6 PUFA's ($\bar{X} \pm \text{SEM}$, $N = 3$) detected in rotifers enriched with five different oil emulsions and a commercial diet, Algamac 2000. Each oil emulsion contained 10% DHA and different levels of ARA (0, 6, 8, 10, 12% total lipid). Treatment means not associated with the same letters are significantly different ($P < 0.05$).

18:2n-6 (Linoleic acid)

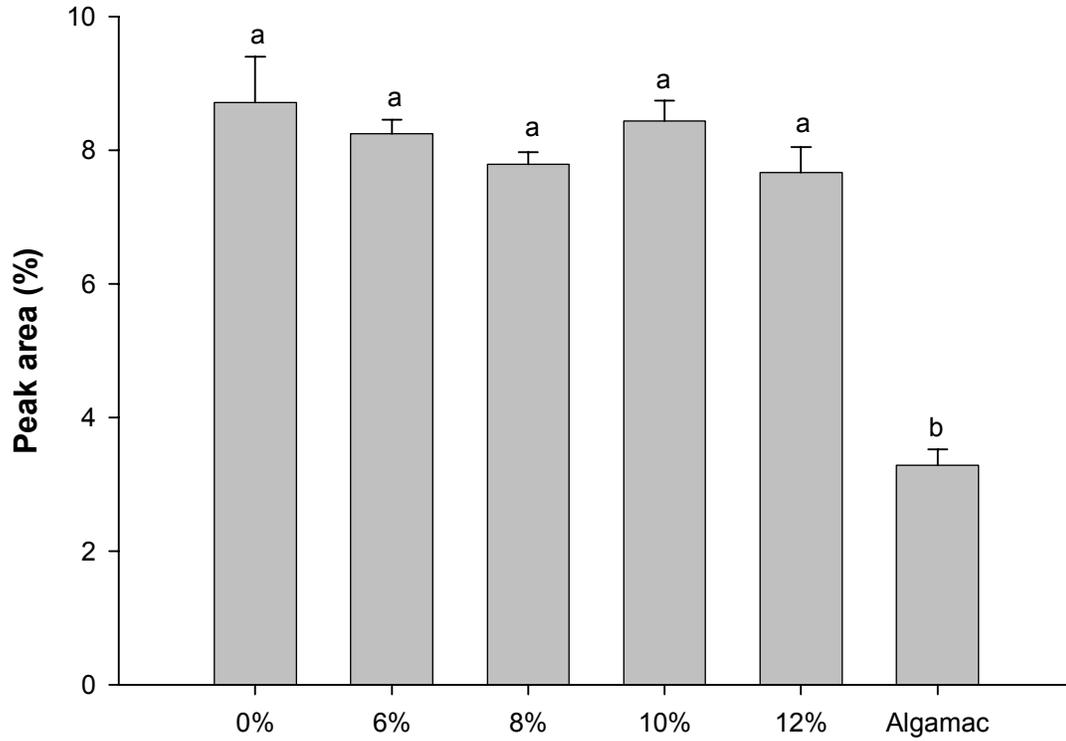


Figure 9. Percentage of linoleic acid, 18:2n-6 ($\bar{X} \pm \text{SEM}$, $N = 3$) detected in rotifers enriched with five different oil emulsions and a commercial diet, Algamac 2000. Each oil emulsion contained 10% DHA and different levels of ARA (0, 6, 8, 10, 12% total lipid). Treatment means not associated with the same letters are significantly different ($P < 0.05$).

20:4n-6 (Arachidonic acid)

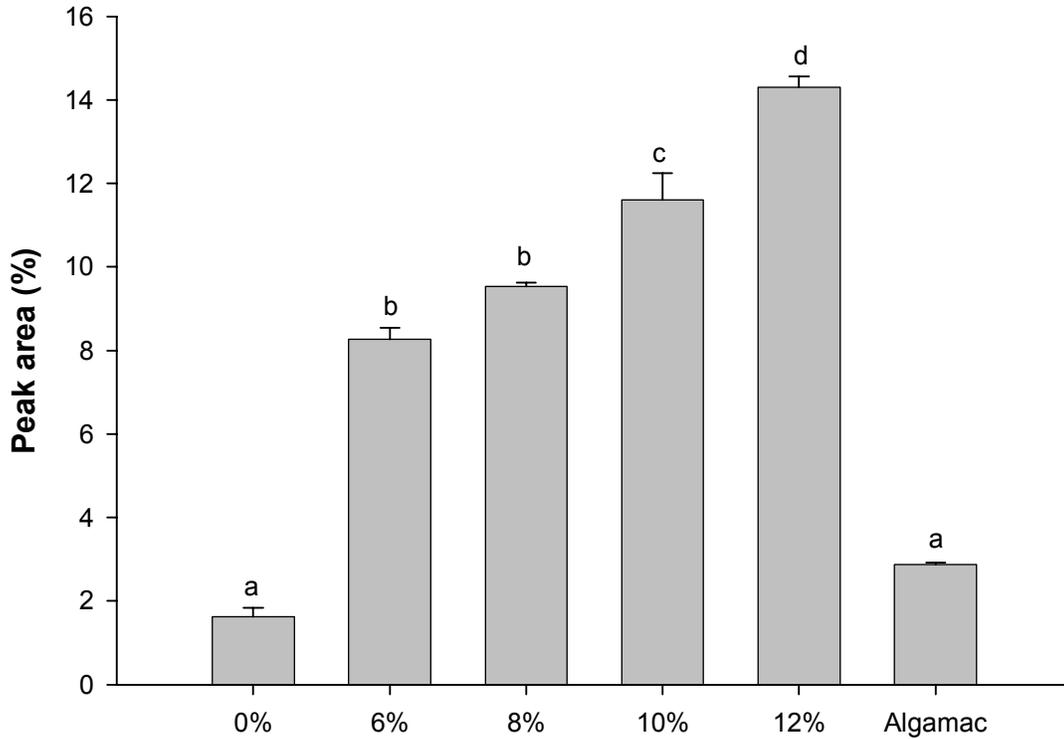


Figure 10. Percentage of arachidonic acid, 20:4n-6 ($\bar{X} \pm \text{SEM}$, $N = 3$) detected in rotifers enriched with five different oil emulsions and a commercial diet, Algamac 2000. Each oil emulsion contained 10% DHA and different levels of ARA (0, 6, 8, 10, 12% total lipid). Treatment means not associated with the same letters are significantly different ($P < 0.05$).

n-3 / n-6 Polyunsaturated fatty acids (PUFA's)

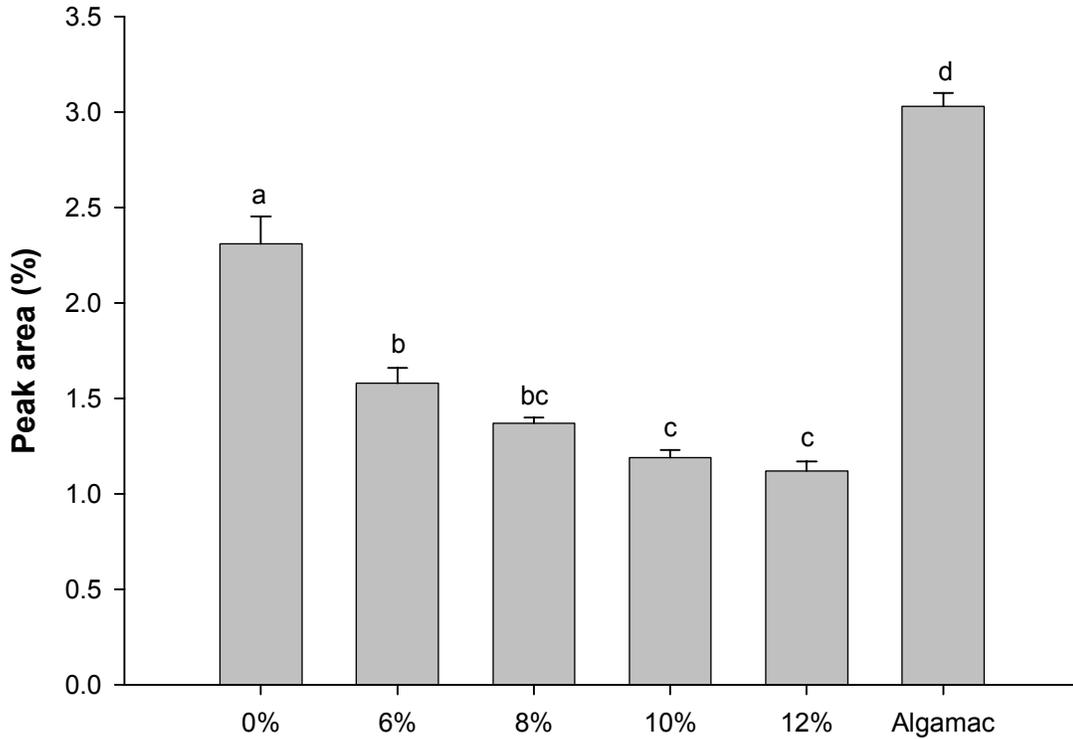


Figure 11. Percentage of n-3/ n-6 polyunsaturated acids, PUFA's ($\bar{X} \pm \text{SEM}$, $N = 3$) detected in rotifers enriched with five different oil emulsions and a commercial diet, Algamac 2000. Each oil emulsion contained 10% DHA and different levels of ARA (0, 6, 8, 10, 12% total lipid). Treatment means not associated with the same letters are significantly different ($P < 0.05$).

20:5n-3/20:4n-6

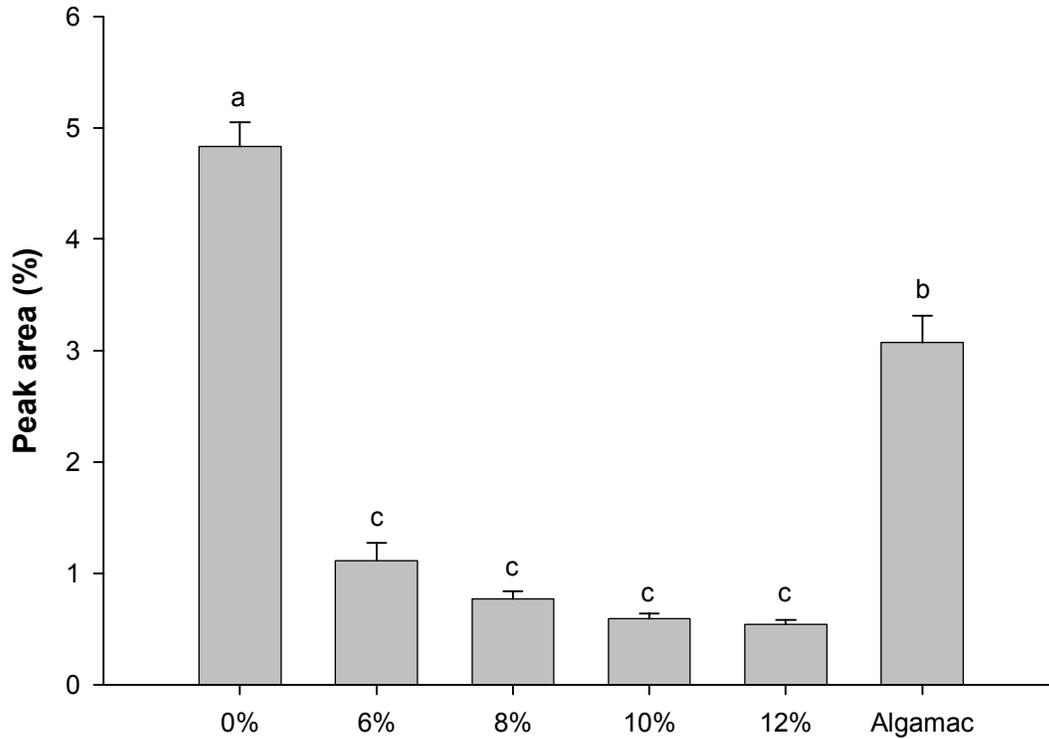


Figure 12. Ratio of 20:5n-3/20:4n-6 ($\bar{X} \pm \text{SEM}$, $N = 3$) detected in rotifers enriched with five different oil emulsions and a commercial diet, Algamac 2000. Each oil emulsion contained 10% DHA and different levels of ARA (0, 6, 8, 10, 12% total lipid). Treatment means not associated with the same letters are significantly different ($P < 0.05$).

22:6n-3/20:4n-6

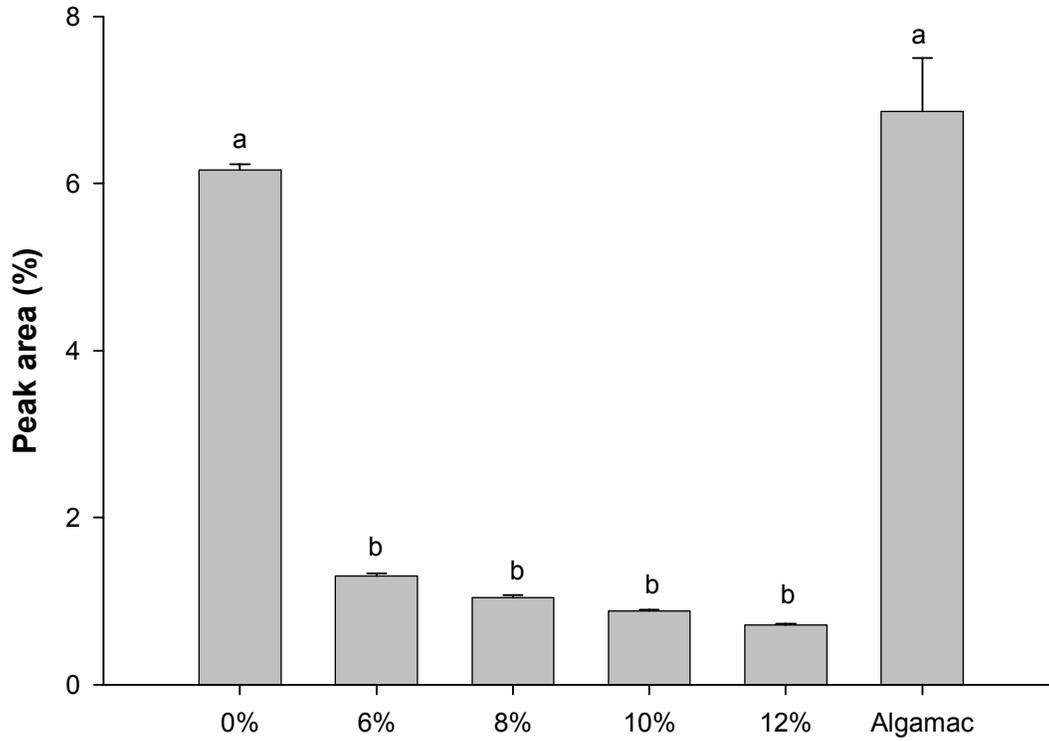


Figure 13. Ratio of 22:6n-3/ 20:4n-6 ($\bar{X} \pm \text{SEM}$, $N = 3$) detected in rotifers enriched with five different oil emulsions and a commercial diet, Algamac 2000. Each oil emulsion contained 10% DHA and different levels of ARA (0, 6, 8, 10, 12% total lipid). Treatment means not associated with the same letters are significantly different ($P < 0.05$).

Fatty Acid Composition of Enriched Artemia

The fatty acid composition of enriched Artemia is reported in Table 2. No significant ($P < 0.05$) treatment effects were observed in total lipid ($\bar{X} = 7.03$) or saturated fatty acids (SFA) ($\bar{X} = 17.7$). Of the SFA's detected myristic acid, 14:0 and palmitic acid, 16:0 showed significant ($P < 0.05$) treatment differences. ALG had higher ($P < 0.05$) levels of 14:0 ($\bar{X} = 3.09$) than the ARA treatments ($\bar{X} = 1.54$). Concentrations of 16:0 were higher ($P < 0.05$) in the ALG treatment ($\bar{X} = 15.7$) than in the ARA treatments ($\bar{X} = 10.5$). No significant ($P > 0.05$) differences were detected in stearic acid, 18:0 ($\bar{X} = 5.48$) in the 0-12% ARA treatments. Levels of 18:0 were undetectable in the ALG treatment (Table 2).

Significant ($P < 0.05$) treatment effects on levels of monounsaturated fatty acids (MUFA) were observed (Fig. 14). A significant ($P < 0.05$) trend toward decreasing MUFA's with increasing ARA was observed from 39.7 at 0% ARA to 24.5 at 12% ARA. MUFA's were significantly ($P < 0.05$) higher in ALG ($\bar{X} = 48.9$) than in the ARA treatments. Of the MUFA's detected, the concentration of 16:1n-7 was higher ($P < 0.05$) in Artemia enriched with ALG ($\bar{X} = 3.98$) than in Artemia enriched with the ARA ($\bar{X} = 2.46 - 3.13$) treatments (Table 2). The concentration of 18:1n-9 was higher ($P < 0.05$) in Artemia enriched with ALG ($\bar{X} = 40.2$) than levels found in Artemia enriched with ARA ($\bar{X} = 20.9 - 34.9$) treatments ($\bar{X} = 20.9$) (Fig. 15). A trend toward decreasing 18:1n-9 with increasing ARA was observed. No treatment ($P > 0.05$) effects were observed for 20:1, eicosenoic acid ($\bar{X} = 1.29$) (Table 2).

The concentration of n-3 PUFA was lower ($P < 0.05$) in the ALG treatment ($\bar{X} = 24.0$) than the ARA treatments ($\bar{X} = 34.9$) (Fig. 16). Of the n-3 PUFA's detected, the

concentration of 18:3n-3 was lower ($P < 0.05$) in the ALG ($\bar{X} = 4.49$) than ARA treatments ($\bar{X} = 22.8$) (Fig. 17). The concentration of 20:5n-3 was higher ($P < 0.05$) in ALG ($\bar{X} = 3.66$) enriched Artemia than the ARA enriched Artemia ($\bar{X} = 1.76$) (Fig. 18). The concentration of 22:6n-3 was higher ($P < 0.05$) in ALG ($\bar{X} = 10.9$) than the ARA treatments ($\bar{X} = 6.09$) (Fig. 19). No significant ($P > 0.05$) treatment effects were observed on the levels of 22:6n-3 / 20:5n-3 ($\bar{X} = 3.40$) (Table 2). There were no significant ($P < 0.05$) treatments effects in 18:4n-3, stearidonic acid ($\bar{X} = 4.38$).

A significant ($P < 0.05$) trend toward increasing n-6 PUFA with increasing ARA was observed, from 7.41 at 0% ARA to 17.4 at 12% ARA. The ALG treatments ($\bar{X} = 9.75$) did not differ significantly ($P > 0.05$) from the 0 and 6% ARA treatments (Fig. 20). Of the n-6 PUFA's detected, the concentration of 18:2n-6 (linoleic acid) was lower ($P < 0.05$) in ALG ($\bar{X} = 4.40$) than in the ARA treatments ($\bar{X} = 6.76$) (Table 2). A significant ($P < 0.05$) trend toward increasing 20:4n-6 (arachidonic acid) with increasing ARA was observed, from 0 at 0% ARA to 10.7 at 12% ARA. Levels of ARA were higher ($P < 0.05$) in the 6, 8, 10 and 12% treatments than the ALG treatment ($\bar{X} = 1.74$) (Fig. 21). Docosapentaenoic acid 22:5n-6 was present in Artemia enriched with ALG ($\bar{X} = 3.92$) and not detected in any of the ARA treatments (Table 2.).

The ratio of n-3/n-6 PUFA's was higher ($P < 0.05$) in the 0% ARA treatment ($\bar{X} = 4.57$) than in the ARA and ALG treatments ($\bar{X} = 2.12 - 2.75$) (Fig. 22). ALG had a higher ($P < 0.05$) ratio of 22:6n-3/20:4n-6 ($\bar{X} = 6.59$) than detected in the ARA treatments ($\bar{X} = 0.81$) (Fig. 23). The ratio of 20:5n-3/20:4n-6 was higher ($P < 0.05$) in the ALG treatment ($\bar{X} = 2.14$) than the ARA treatments ($\bar{X} = 0.22$) (Fig. 24).

Table 2. Fatty acid composition (% of total FAMES) of enriched *Artemia* nauplii. Values represent means \pm SEM, (N=3).

Enriched diets received by larvae (% total lipid as ARA and Algamac 2000)						
Fatty acids	0%	6%	8%	10%	12%	Algamac 2000
14:0	1.48 \pm 0.06 a	1.49 \pm 0.09 a	1.68 \pm 0.15 a	1.66 \pm 0.08 a	1.42 \pm 0.09 a	3.09 \pm 0.14 b
16:0	10.2 \pm 0.19 a	10.5 \pm 0.08 a	10.8 \pm 0.39 a	10.5 \pm 0.28 a	10.4 \pm 0.01 a	15.7 \pm 0.55 b
16:1n-7	3.13 \pm 0.01 a	2.77 \pm 0.20 ab	2.62 \pm 0.14 ab	2.57 \pm 0.19 ab	2.46 \pm 0.06 b	3.98 \pm 0.11 c
18:0	5.08 \pm 0.29	5.55 \pm 0.15	5.39 \pm 0.14	5.42 \pm 0.17	5.99 \pm 0.23	nd
18:1n-9	34.9 \pm 2.15 ab	28.9 \pm 1.58 bc	26.5 \pm 1.06 cd	24.1 \pm 0.51 cd	20.9 \pm 0.67 d	40.2 \pm 0.94 a
18:2n-6	7.41 \pm 0.48 a	6.64 \pm 0.03 a	6.64 \pm 0.05 a	6.62 \pm 0.02 a	6.49 \pm 0.07 a	4.40 \pm 0.14 b
18:3n-3	22.4 \pm 1.48 a	22.0 \pm 1.65 a	22.2 \pm 1.53 a	22.6 \pm 1.12 a	24.7 \pm 1.45 a	4.49 \pm 0.21 b
18:4n-3	4.33 \pm 0.30	3.98 \pm 0.34	4.27 \pm 0.35	4.44 \pm 0.45	4.31 \pm 0.27	4.98 \pm 0.34
20:1	1.61 \pm 0.22	1.65 \pm 0.31	1.05 \pm 0.05	1.11 \pm 0.03	1.19 \pm 0.05	1.15 \pm 0.02
20:4n-6	0	5.9 \pm 0.41 a	7.52 \pm 0.73 a	9.09 \pm 1.04 ab	10.9 \pm 0.73 b	1.74 \pm 0.29 c
20:5n-3	1.79 \pm 0.09 a	1.80 \pm 0.07 a	1.80 \pm 0.08 a	1.63 \pm 0.15 a	1.79 \pm 0.13 a	3.66 \pm 0.45 b
22:5n-6	0	0	0	0	0	3.92 \pm 0.52
22:6n-3	5.06 \pm 0.30 a	6.55 \pm 0.49 a	6.46 \pm 0.68 a	6.60 \pm 0.54 a	5.80 \pm 0.51 a	10.9 \pm 1.23 b
Σ Saturates	16.8 \pm 0.19	17.5 \pm 0.13	17.8 \pm 0.67	17.5 \pm 0.31	17.8 \pm 0.20	18.8 \pm 0.69
Σ Monounsaturates	39.7 \pm 2.20 a	33.3 \pm 1.15 b	30.1 \pm 1.00 bc	27.8 \pm 0.45 cd	24.5 \pm 0.59 d	48.9 \pm 0.71 e
Σ n-3 Polyunsaturates	33.6 \pm 1.39a	34.3 \pm 1.51 a	34.7 \pm 1.55 a	35.3 \pm 1.04 a	36.6 \pm 1.31 a	24.0 \pm 1.16 b
Σ n-6 Polyunsaturates	7.41 \pm 0.48 a	12.6 \pm 0.39 bc	14.2 \pm 0.69 c	15.7 \pm 1.02 cd	17.4 \pm 0.75 d	9.75 \pm 0.49 ab
n-3/n-6 Polyunsaturates	4.57 \pm 0.32 a	2.75 \pm 0.20 b	2.47 \pm 0.18 b	2.27 \pm 0.19 b	2.12 \pm 0.15 b	2.46 \pm 0.18 b
DHA/EPA	2.83 \pm 0.12	3.64 \pm 0.29	3.58 \pm 0.29	4.05 \pm 0.21	3.27 \pm 0.37	3.05 \pm 0.46
DHA/ARA	0	1.10 \pm 0.03 a	0.85 \pm 0.02 a	0.73 \pm 0.03 a	0.53 \pm 0.03 a	6.59 \pm 1.17 b
EPA/ARA	0	0.31 \pm 0.02 a	0.24 \pm 0.02 a	0.18 \pm 0.01 a	0.17 \pm 0.01 a	2.14 \pm 0.09 b
Total Lipid	6.36 \pm 0.20	6.26 \pm 0.29	8.06 \pm 0.92	7.1 \pm 0.15	6.90 \pm 0.23	7.48 \pm 0.39

(% Wet tissue Weight)

Mean values within rows that are not followed by the same letter are significantly ($P < 0.05$) different.

Monounsaturated fatty acids (MUFA's)

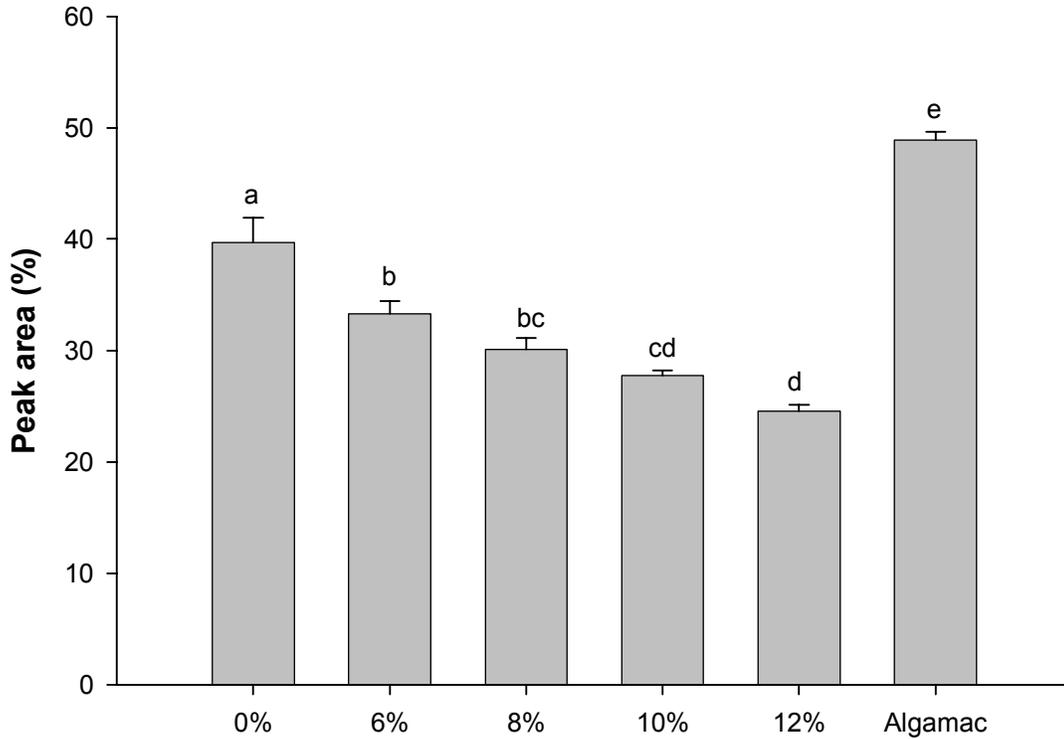


Figure 14. Percentage of monounsaturated fatty acids, MUFA's ($\bar{X} \pm \text{SEM}$, $N = 3$) detected in *Artemia* enriched with five different oil emulsions and a commercial diet, Algamac 2000. Each oil emulsion contained 10% DHA and different levels of ARA (0, 6, 8, 10, 12% total lipid). Treatment means not associated with the same letters are significantly different ($P < 0.05$).

18:1n-9 (Oleic acid)

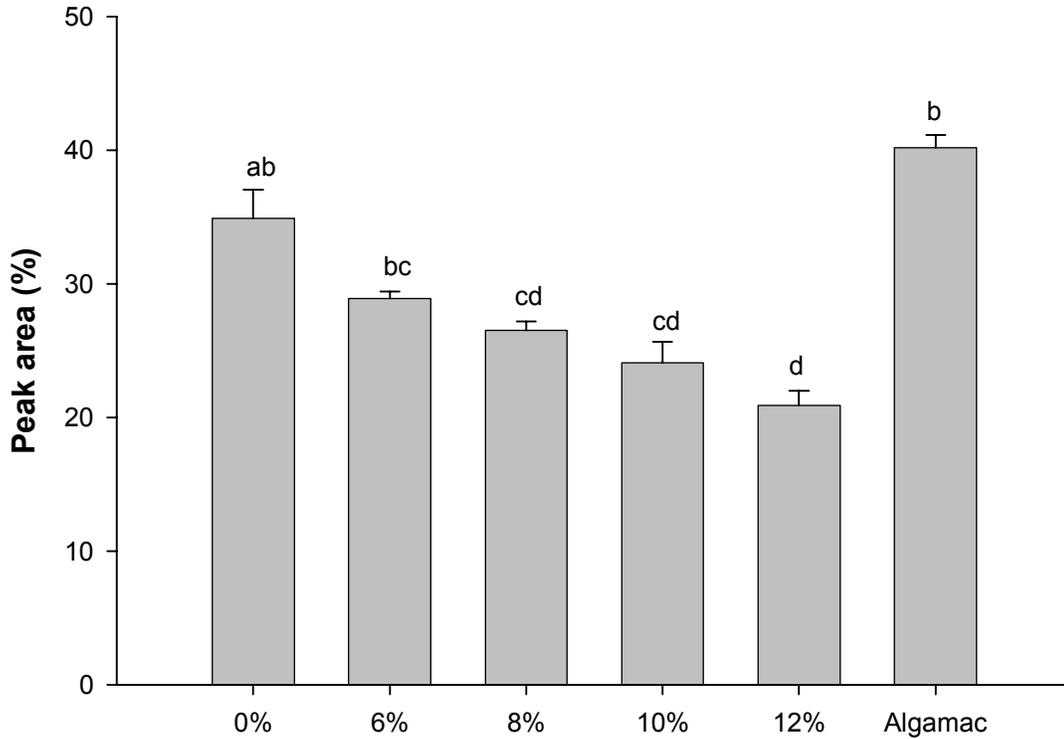


Figure 15. Percentage of oleic acid, 18:1n-9 ($\bar{X} \pm \text{SEM}$, $N = 3$) detected in Artemia enriched with five different oil emulsions and a commercial diet, Algamac 2000. Each oil emulsion contained 10% DHA and different levels of ARA (0, 6, 8, 10, 12% total lipid). Treatment means not associated with the same letters are significantly different ($P < 0.05$).

n-3 Polyunsaturated fatty acids (PUFA's)

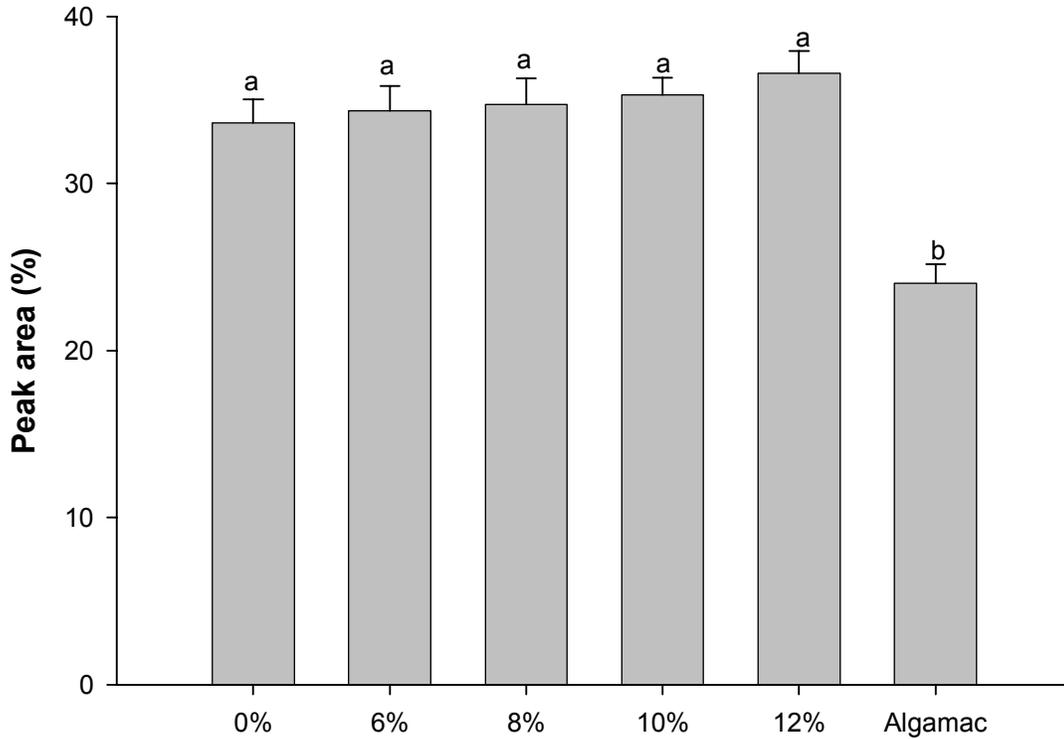


Figure 16. Percentage of n-3 polyunsaturated fatty acids (PUFA's) ($\bar{X} \pm \text{SEM}$, $N = 3$) detected in Artemia enriched with five different oil emulsions and a commercial diet, Algamac 2000. Each oil emulsion contained 10% DHA and different levels of ARA (0, 6, 8, 10, 12% total lipid). Treatment means not associated with the same letters are significantly different ($P < 0.05$).

18:3n-3 (Linolenic acid)

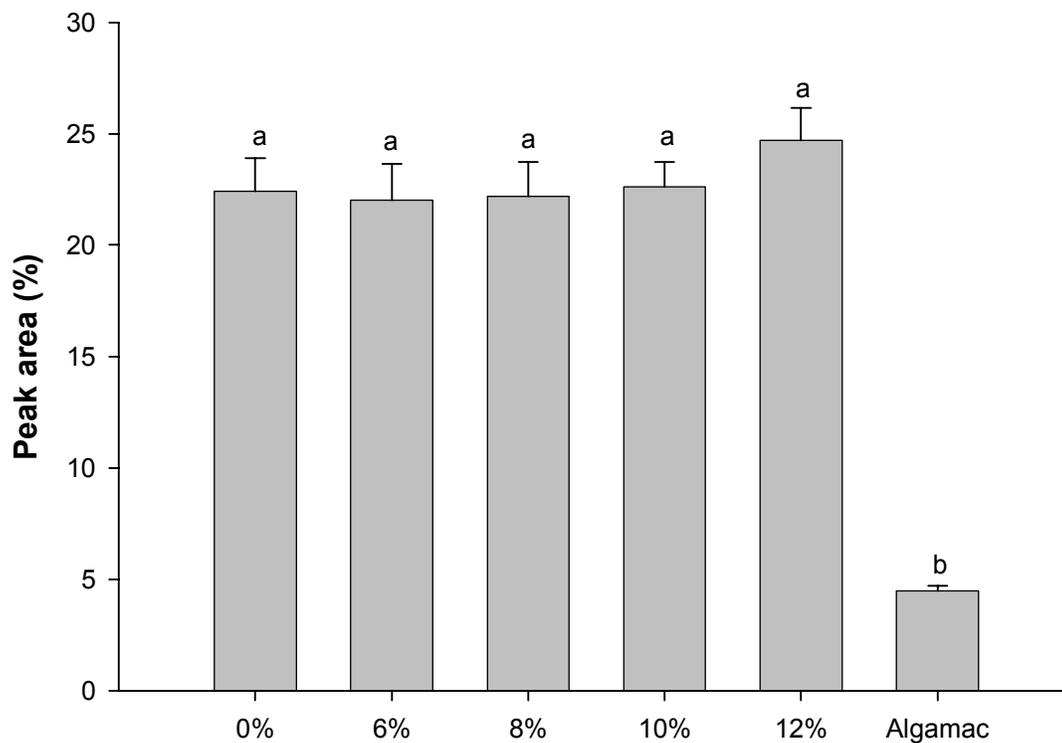


Figure 17. Percentage of linolenic acid, 18:3n-3 ($\bar{X} \pm \text{SEM}$, $N = 3$) detected in *Artemia* enriched with five different oil emulsions and a commercial diet, Algamac 2000. Each oil emulsion contained 10% DHA and different levels of ARA (0, 6, 8, 10, 12% total lipid). Treatment means not associated with the same letters are significantly different ($P < 0.05$).

20:5n-3 Eicosapentaenoic acid

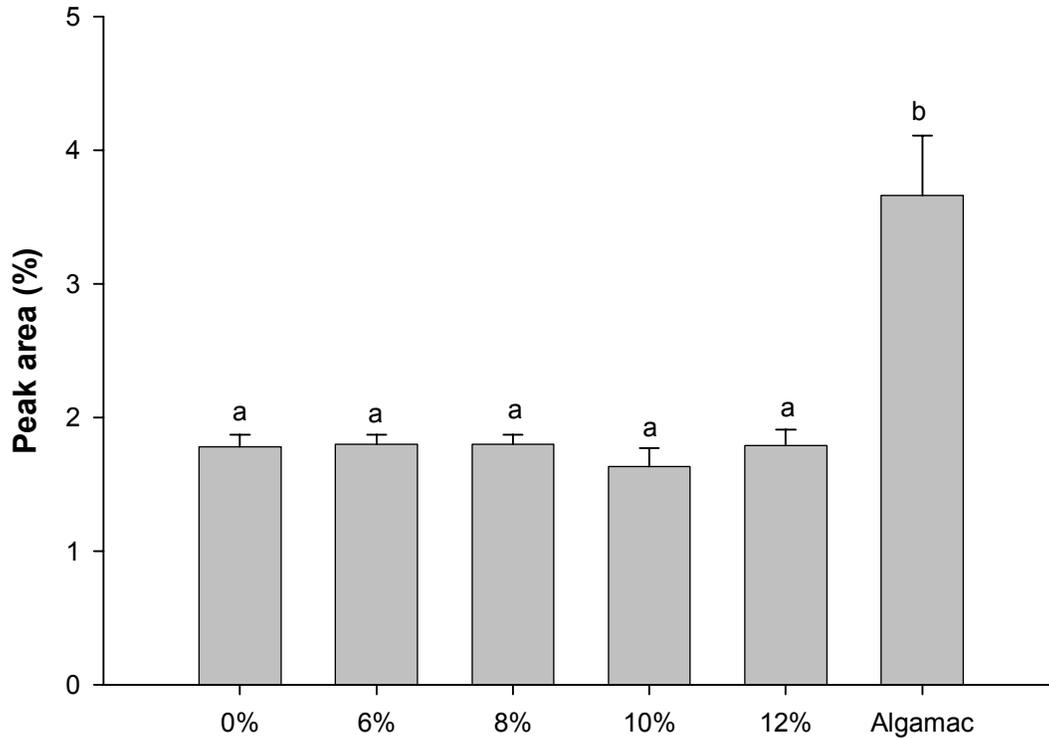


Figure 18. Percentage of eicosapentaenoic acid, 20:5n-3 ($\bar{X} \pm \text{SEM}$, $N = 3$) detected in Artemia enriched with five different oil emulsions and a commercial diet, Algamac 2000. Each oil emulsion contained 10% DHA and different levels of ARA (0, 6, 8, 10, 12% total lipid). Treatment means not associated with the same letters are significantly different ($P < 0.05$).

22:6n-3 (Docosahexaenoic acid)

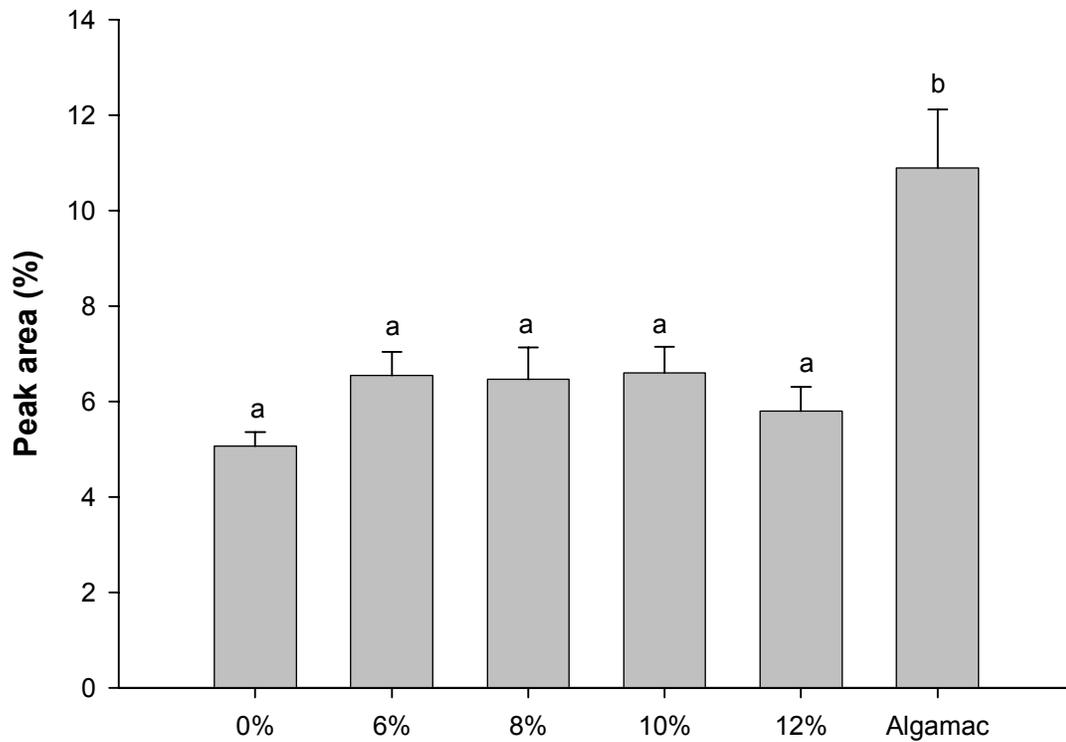


Figure 19. Percentage of docosahexaenoic acid, 22:6n-3 ($\bar{X} \pm \text{SEM}$, $N=3$) detected in Artemia enriched with five different oil emulsions and a commercial diet, Algamac 2000. Each oil emulsion contained 10% DHA and different levels of ARA (0, 6, 8, 10, 12% total lipid). Treatment means not associated with the same letters are significantly different ($P < 0.05$).

n-6 Polyunsaturated fatty acid (PUFA)

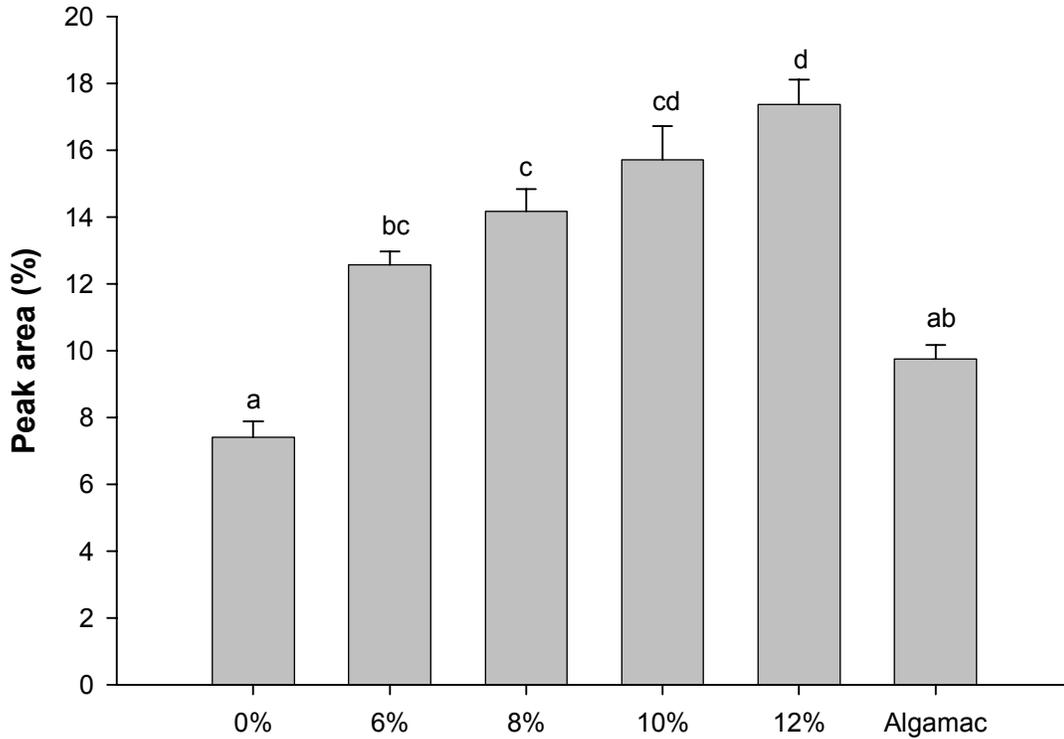


Figure 20. Percentage of n-6 PUFA's ($\bar{X} \pm \text{SEM}$, $N=3$) detected in Artemia enriched with five different oil emulsions and a commercial diet, Algamac 2000. Each oil emulsion contained 10% DHA and different levels of ARA (0, 6, 8, 10, 12% total lipid). Treatment means not associated with the same letters are significantly different ($P < 0.05$).

20:4n-6 (Arachidonic acid)

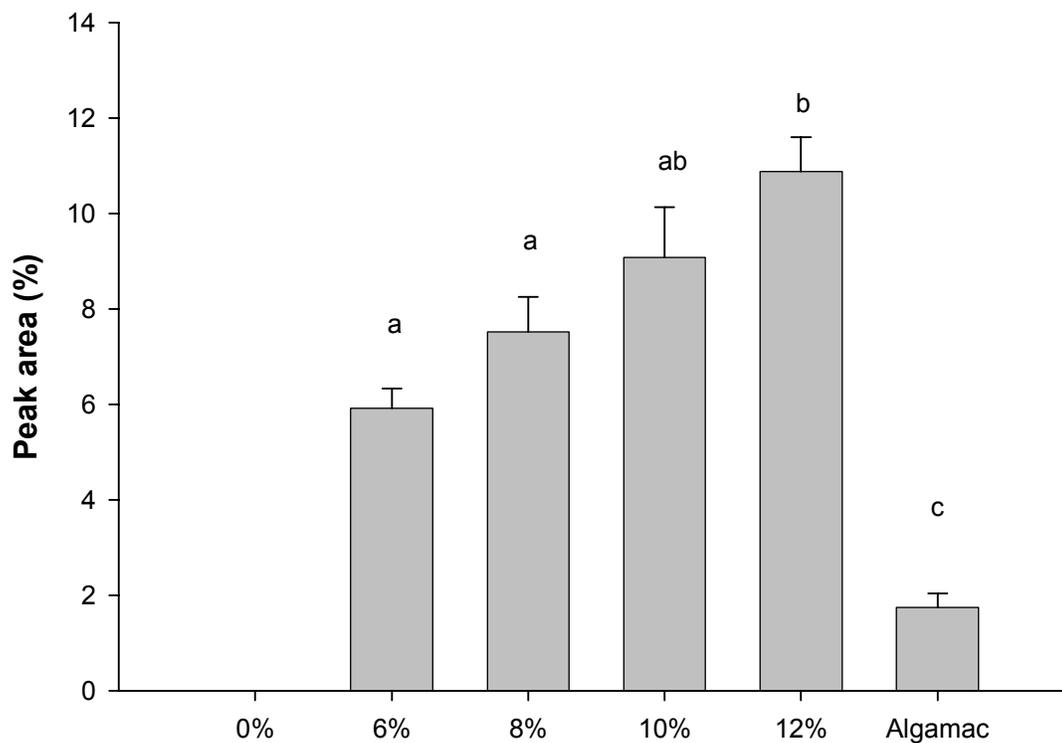


Figure 21. Percentage of arachidonic acid, 20:4n-6 ($\bar{X} \pm \text{SEM}$, $N = 3$) detected in Artemia enriched with five different oil emulsions and a commercial diet, Algamac 2000. Each oil emulsion contained 10% DHA and different levels of ARA (0, 6, 8, 10, 12% total lipid). Treatment means not associated with the same letters are significantly different ($P < 0.05$).

n-3 / n-6 Polyunsaturated fatty acids (PUFA's)

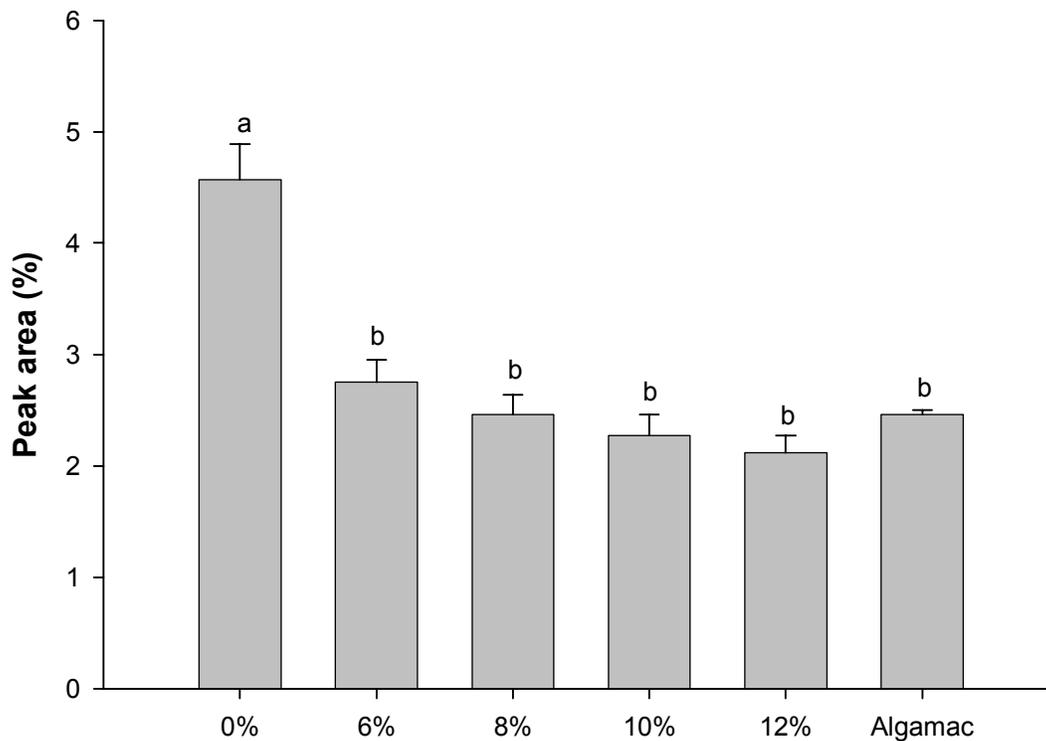


Figure 22. The ratio of n-3/n-6 PUFA ($\bar{X} \pm \text{SEM}$, $N = 3$) detected in *Artemia* enriched with five different oil emulsions and a commercial diet, Algamac 2000. Each oil emulsion contained 10% DHA and different levels of ARA (0, 6, 8, 10, 12% total lipid). Treatment means not associated with the same letters are significantly different ($P < 0.05$).

22:6n-3/20:4n-6

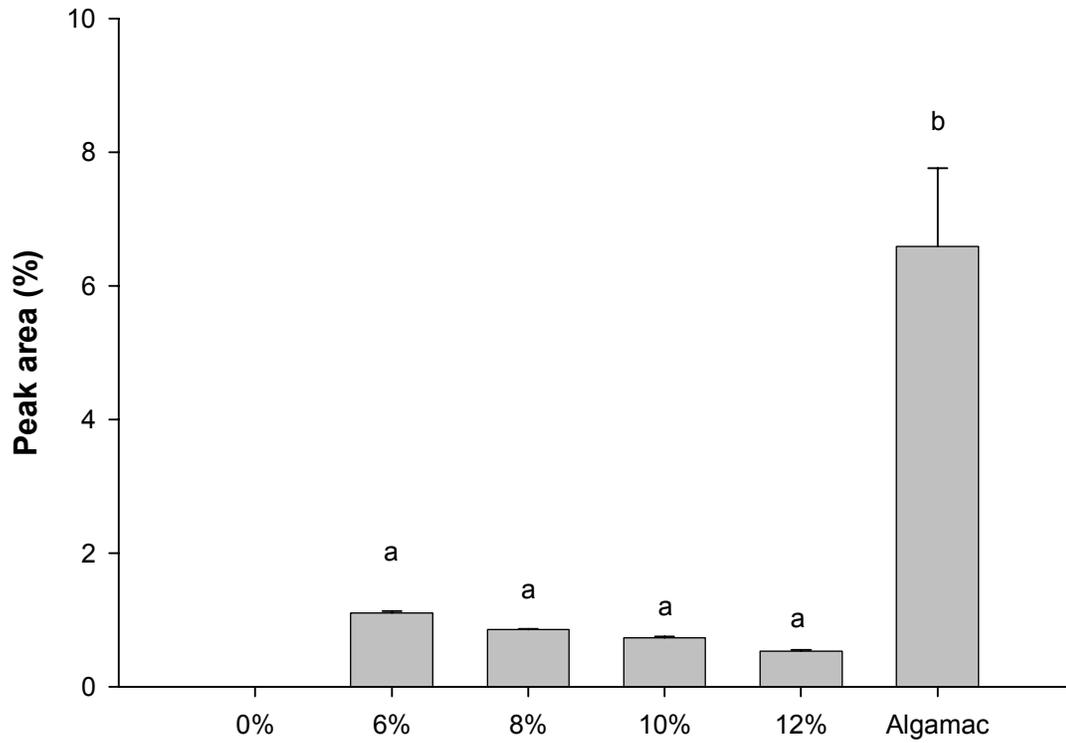


Figure 23. The ratio of 22:6n-3/20:4n-3, DHA/ARA ($\bar{X} \pm \text{SEM}$, $N = 3$) detected in *Artemia* enriched with five different oil emulsions and a commercial diet, Algamac 2000. Each oil emulsion contained 10% DHA and different levels of ARA (0, 6, 8, 10, 12% total lipid). Treatment means not associated with the same letters are significantly different ($P < 0.05$).

20:5n-3/20:4n-6

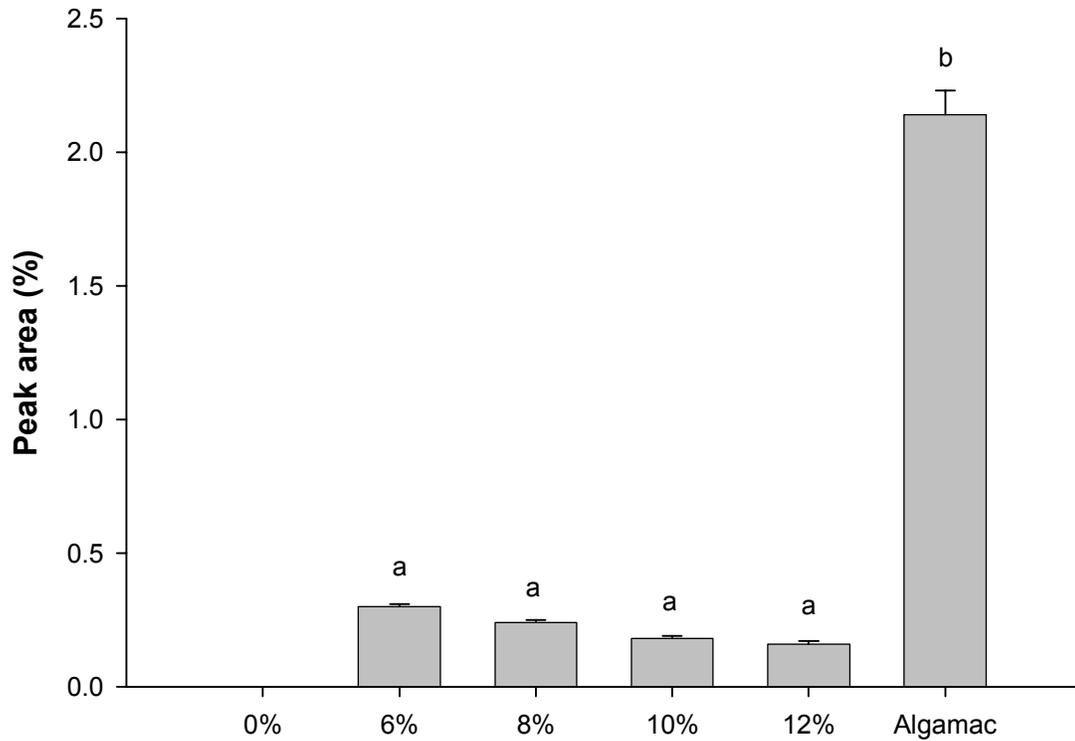


Figure 24. The ratio of 20:5n-3/20:4n-6, EPA/ARA ($\bar{X} \pm \text{SEM}$, $N = 3$) detected in Artemia enriched with five different oil emulsions and a commercial diet, Algamac 2000. Each oil emulsion contained 10% DHA and different levels of ARA (0, 6, 8, 10, 12% total lipid). Treatment means not associated with the same letters are significantly different ($P < 0.05$).

Fatty Acid Composition of Larval Black Sea Bass

The fatty acid composition of black sea bass larvae is reported in Table 3. No significant ($P < 0.05$) treatment effects were observed in total lipid ($\bar{X} = 5.48$). Of the saturated fatty acids (SFA's) detected, 14:0 (myristic acid) was higher ($P < 0.05$) in the 10% ARA ($\bar{X} = 1.03$) treatment than the 6, 8, 12% ($\bar{X} = 0.71 - 0.75$) ARA treatments but was not different from the 0% ARA ($\bar{X} = 0.79$) and ALG treatments ($\bar{X} = 0.83$). Concentrations of 16:0 were significantly ($P < 0.05$) higher in the ALG treatment ($\bar{X} = 14.9$) than the 6 and 8% ARA treatments ($\bar{X} = 13.2 - 13.4$). There were no significant ($P > 0.05$) treatment effects on the concentrations of stearic acid, 18:0 ($\bar{X} = 8.16$).

A significant ($P < 0.05$) trend of decreasing MUFA's with increasing ARA was observed from 31.2 TFA in 0% ARA to 22.4 TFA in 12% ARA (Fig. 25). The ALG treatment ($\bar{X} = 18.1$) was significantly ($P < 0.05$) lower than the ARA treatments (Fig. 25). Of the MUFA's detected, the concentration of 16:1n-7 was higher ($P < 0.05$) in larvae fed ALG ($\bar{X} = 2.96$) than in rotifers fed 0, 6, 8 and 12% ARA ($\bar{X} = 2.42$) but not significantly different ($P > 0.05$) from larvae fed 10% ARA ($\bar{X} = 2.93$) (Table 3). A trend ($P < 0.05$) of decreasing 18:1n-9 with increasing ARA was observed from 26.3 TFA in 0% ARA to 18.3 TFA in 12% ARA. The ALG treatment ($\bar{X} = 13.3$) was lower ($P < 0.05$) than the ARA treatments (Fig. 26). No treatment ($P > 0.05$) effects were observed in 20:1, eicosenoic acid ($\bar{X} = 1.93$) (Table 3).

Significant ($P < 0.05$) treatment effects were observed in the concentrations of n-3 polyunsaturated fatty acids (PUFA's) (Fig. 27). The concentration of n-3 PUFA was higher ($P < 0.05$) in the ALG treatment ($\bar{X} = 43.9$) than in the ARA treatments ($\bar{X} = 34.9$). Of the n-3 PUFA's detected, the concentration of 18:3n-3 was higher ($P < 0.05$) in

ALG ($\bar{X} = 22.5$) than 0, 8 and 10% ARA treatments ($\bar{X} = 19.3$) but was not different ($P > 0.05$) from the 6 and 12% ARA treatments ($\bar{X} = 20.7$) (Fig. 28). There was no significant ($P > 0.05$) treatment effect observed in 18:4n-3, stearidonic acid ($\bar{X} = 3.21$) (Table 3). Larvae fed prey enriched with ALG contained higher ($P < 0.05$) levels of 20:5n-3 ($\bar{X} = 4.76$) than the 6-12% ARA treatments ($\bar{X} = 3.07$). The 0% ARA treatment had lower ($P < 0.05$) levels of 20:5n-3 ($\bar{X} = 3.89$) than the ALG fed larvae but higher ($P < 0.05$) than the 6, 8, 10 and 12% ARA treatments (Fig. 29). The concentration of 22:5n-3 was higher ($P < 0.05$) in the 0, 8 and 10% ARA ($\bar{X} = 1.29$) fed larvae than the ALG ($\bar{X} = 0.91$) fed larvae (Fig. 30). The concentration of 22:6n-3 was higher ($P < 0.05$) in ALG ($\bar{X} = 12.6$) than the ARA treatments ($\bar{X} = 7.45$) (Fig. 31). Larvae fed 12% ARA had a higher ($P < 0.05$) ratio of 22:6n-3/20:5n-3 ($\bar{X} = 2.71$) than larvae fed 0, 6 and 8% ARA ($\bar{X} = 2.11$) (Fig. 32). Larvae fed ALG (3.48) showed no significant ($P > 0.05$) differences in the ratio of 22:6n-3/20:4n-6 from those fed ARA ($\bar{X} = 0.75 - 4.92$) (Table 3.).

A significant ($P < 0.05$) trend of increasing n-6 PUFA's with increasing ARA was observed from 8.42 at 0% ARA to 15.9 at 12% ARA. The ALG treatment (11.5%) was lower ($P < 0.05$) than 6 – 12% ARA, but higher ($P < 0.05$) than 0% ARA (Fig. 33). A trend of decreasing 18:2n-6 with increasing ARA was observed from 6.77 TFA in 0% ARA to 5.73 TFA in 12% ARA (Fig. 34). The ALG treatment ($\bar{X} = 4.53$) had lower ($P < 0.05$) levels of 18:2n-6 than the ARA treatments. A trend of increasing 20:4n-6 with increasing ARA was observed from 1.64 in 0% ARA to 10.2 in the 12% ARA treatment (Fig. 35). There was more ($P < 0.05$) 20:4n-6 detected in the ALG treatment ($\bar{X} = 3.61$) than the 0% ARA treatment ($\bar{X} = 1.64$) but less ($P < 0.05$) than the 6 – 12% ARA treatment ($\bar{X} = 6.73 - 10.2$). Docosapentaenoic acid 22:5n-6 was detected in larvae fed

prey enriched with ALG ($\bar{X} = 3.16$) and was not found in any of the ARA treatments (Table 3.).

The ratio of n-3/n-6 PUFA was higher ($P < 0.05$) in the 0% ARA fed larvae ($\bar{X} = 4.20$) than the 6, 8, 10, 12% ARA ($\bar{X} = 2.72 - 2.21$) and ALG treatments ($\bar{X} = 3.83$). The ratio of n-3/n-6 PUFA detected in ALG was significantly ($P < 0.05$) higher than detected in the 6 – 12% ARA treatments (Fig. 36). The ALG fed larvae had a higher ($P < 0.05$) ratio of 22:6n-3/20:4n-6 ($\bar{X} = 3.48$) than the 6, 8, 10 and 12% ARA treatments ($\bar{X} = 0.86$). The 0% ARA fed larvae had a higher ($P < 0.05$) ratio of 22:6n-3/ 20:4n-6 ($\bar{X} = 4.92$) than the ALG treatment or ARA treatments (Fig. 37). The ALG fed larvae had a higher ($P < 0.05$) ratio of 20:5n-3/20:4n-6 ($\bar{X} = 1.32$) than those detected in the 6, 8, 10 and 12% ARA treatments ($\bar{X} = 0.37$) (Fig. 38). The 0% ARA fed larvae had a higher ($P < 0.05$) ratio of 20:5n-3/ 20:4n-6 ($\bar{X} = 2.3$) than the ALG treatment.

Table 3. Fatty acid composition (% of total FAMES) of black sea bass *Centropristis striata* larvae (day 24 post hatching). Values represent means \pm SEM, (N=3).

Enriched diets received by larvae (% total lipid as ARA and Algamac 2000)						
Fatty acids	0%	6%	8%	10%	12%	Algamac 2000
14:0	0.79 \pm 0.01 ab	0.75 \pm 0.02 b	0.71 \pm 0.02 b	1.03 \pm 0.12 a	0.73 \pm 0.02 b	0.83 \pm 0.05 ab
16:0	13.8 \pm 0.27 ab	13.4 \pm 0.31 b	13.2 \pm 0.05 b	14.2 \pm 0.39 ab	13.5 \pm 0.08 b	14.9 \pm 0.34 a
16:1n-7	2.55 \pm 0.02 b	2.52 \pm 0.06 b	2.29 \pm 0.01 b	2.93 \pm 0.32 ab	2.31 \pm 0.10 b	2.96 \pm 0.12 a
18:0	8.49 \pm 0.27	7.79 \pm 0.12	8.41 \pm 0.04	7.63 \pm 0.48	8.60 \pm 0.28	8.01 \pm 0.61
18:1n-9	26.3 \pm 0.28 a	23.0 \pm 0.17 b	21.4 \pm 0.25 c	19.2 \pm 0.36 d	18.3 \pm 0.12 d	13.3 \pm 0.41 e
18:2n-6	6.77 \pm 0.08 a	6.39 \pm 0.06 b	5.97 \pm 0.01 c	6.09 \pm 0.12 bc	5.73 \pm 0.08 c	4.53 \pm 0.09 d
18:3n-3	19.0 \pm 0.40 a	21.2 \pm 0.42 ab	19.3 \pm 0.31 a	19.5 \pm 0.66 a	20.1 \pm 0.35 ab	22.5 \pm 1.17 b
18:4n-3	4.33 \pm 0.30	3.98 \pm 0.34	4.27 \pm 0.35	4.44 \pm 0.45	4.31 \pm 0.27	4.98 \pm 0.34
20:1	2.33 \pm 0.09	1.71 \pm 0.31	2.19 \pm 0.11	1.61 \pm 0.32	1.85 \pm 0.17	1.91 \pm 0.22
20:4n-6	1.64 \pm 0.04 a	6.73 \pm 0.18 c	7.87 \pm 0.09 d	9.32 \pm 0.23 e	10.2 \pm 0.19 f	3.61 \pm 0.02 b
20:5n-3	3.89 \pm 0.09 b	3.25 \pm 0.13 c	3.15 \pm 0.04 c	3.06 \pm 0.02 c	2.84 \pm 0.13 c	4.76 \pm 0.25 a
22:5n-3	1.28 \pm 0.04 a	1.12 \pm 0.09 ab	1.26 \pm 0.03 a	1.33 \pm 0.09 a	1.17 \pm 0.07 ab	0.91 \pm 0.05 b
22:5n-6	0	0	0	0	0	3.16 \pm 0.11
22:6n-3	8.07 \pm 0.52 a	6.79 \pm 0.21 a	6.72 \pm 0.11 a	8.06 \pm 0.42 a	7.64 \pm 0.31 a	12.6 \pm 0.57 b
Σ Saturates	23.0 \pm 0.54	21.9 \pm 0.36	22.3 \pm 0.07	22.9 \pm 0.51	22.8 \pm 0.23	23.8 \pm 0.88
Σ Monounsaturates	31.2 \pm 0.22 a	27.3 \pm 0.19 b	25.9 \pm 0.34 c	23.8 \pm 0.37 d	22.4 \pm 0.16 d	18.1 \pm 0.46 e
Σ n-3 Polyunsaturates	35.4 \pm 0.02 a	35.7 \pm 0.41 a	33.7 \pm 0.32 a	35.0 \pm 0.59 a	35.1 \pm 0.32 a	43.9 \pm 1.18 b
Σ n-6 Polyunsaturates	8.42 \pm 0.05 a	13.1 \pm 0.21 c	13.8 \pm 0.09 d	15.4 \pm 0.21 e	15.9 \pm 0.13 e	11.5 \pm 0.11 b
n-3/n-6 Polyunsaturates	4.20 \pm 0.03 a	2.72 \pm 0.04 c	2.44 \pm 0.03 d	2.27 \pm 0.03 d	2.21 \pm 0.03 d	3.83 \pm 0.12 b
DHA/EPA	2.09 \pm 0.18 a	2.10 \pm 0.10 ab	2.13 \pm 0.05 ab	2.63 \pm 0.13 bc	2.71 \pm 0.21 c	2.67 \pm 0.25 abc
DHA/ARA	4.92 \pm 0.33 a	1.01 \pm 0.02 c	0.85 \pm 0.01 c	0.86 \pm 0.03 c	0.75 \pm 0.02 c	3.48 \pm 0.16 b
EPA/ARA	2.37 \pm 0.06 a	0.48 \pm 0.01 c	0.41 \pm 0.01 cd	0.33 \pm 0.01 cd	0.28 \pm 0.02 d	1.32 \pm 0.07 b
Total Lipid	5.58 \pm 0.19	6.13 \pm 0.09	5.11 \pm 0.31	5.70 \pm 0.32	5.25 \pm 0.36	5.13 \pm 0.33

(% Wet tissue Weight)

Mean values within rows that are not followed by the same letter are significantly ($P < 0.05$) different.

Monounsaturated fatty acids (MUFA's)

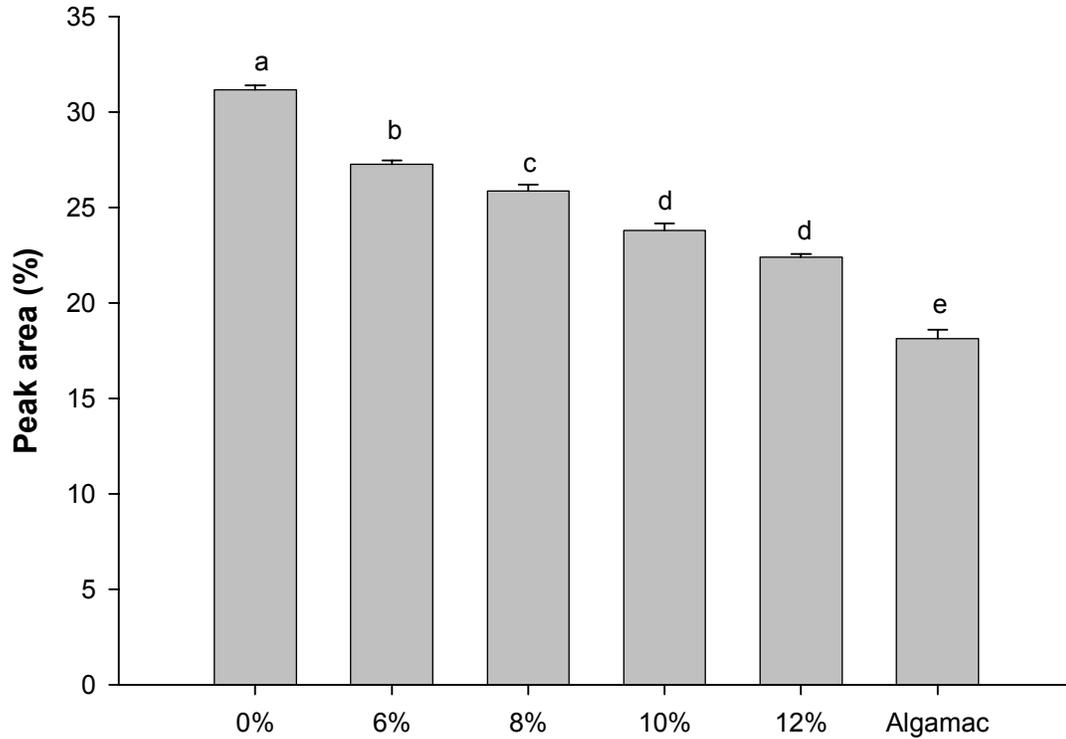


Figure 25. Percentage of monounsaturated fatty acids, MUFA's ($\bar{X} \pm \text{SEM}$, $N = 3$) detected in black sea bass larvae fed live prey enriched with five different oil emulsions and a commercial diet, Algamac 2000. Each oil emulsion contained 10% DHA and different levels of ARA (0, 6, 8, 10, 12% total lipid). Treatment means not associated with the same letters are significantly different ($P < 0.05$).

18:1n-9 (Oleic acid)

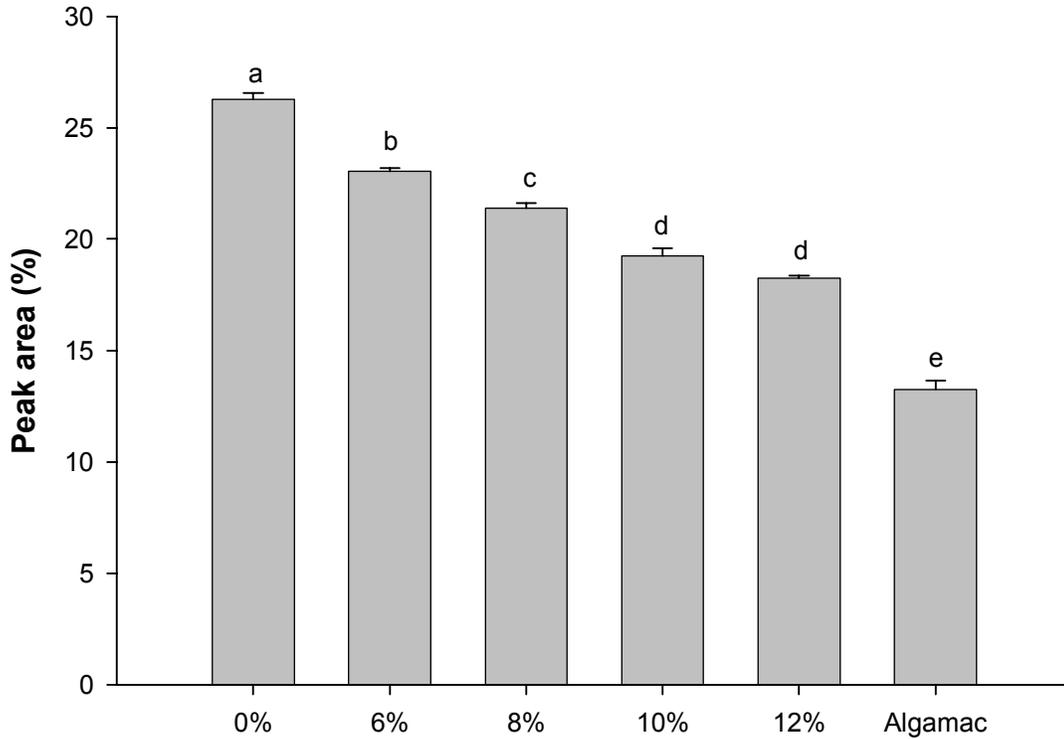


Figure 26. Percentage of oleic acid, 18:1n-9 ($\bar{X} \pm \text{SEM}$, $N = 3$) detected in black sea bass larvae fed live prey enriched with five different oil emulsions and a commercial diet, Algamac 2000. Each oil emulsion contained 10% DHA and different levels of ARA (0, 6, 8, 10, 12% total lipid). Treatment means not associated with the same letters are significantly different ($P < 0.05$).

n-3 Polyunsaturated fatty acids (PUFA's)

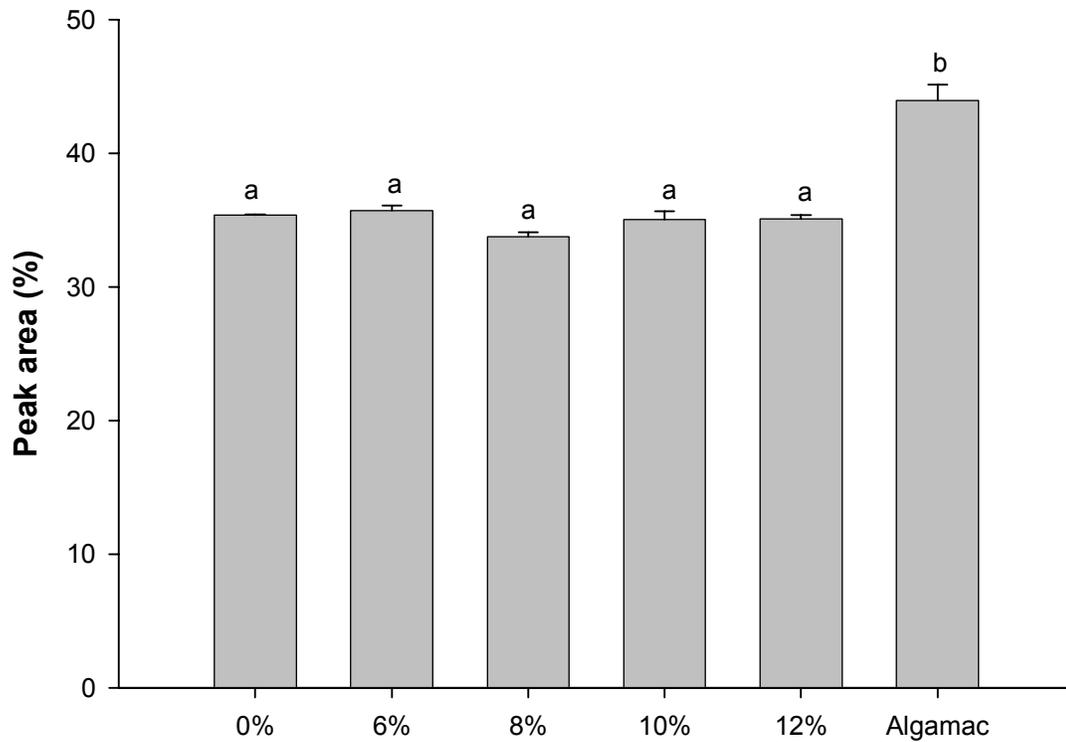


Figure 27. Percentage of n-3 polyunsaturated fatty acids (PUFA's) ($\bar{X} \pm \text{SEM}$, $N = 3$) detected in black sea bass larvae fed live prey enriched with five different oil emulsions and a commercial diet, Algamac 2000. Each oil emulsion contained 10% DHA and different levels of ARA (0, 6, 8, 10, 12% total lipid). Treatment means not associated with the same letters are significantly different ($P < 0.05$).

18:3n-3 (Linolenic acid)

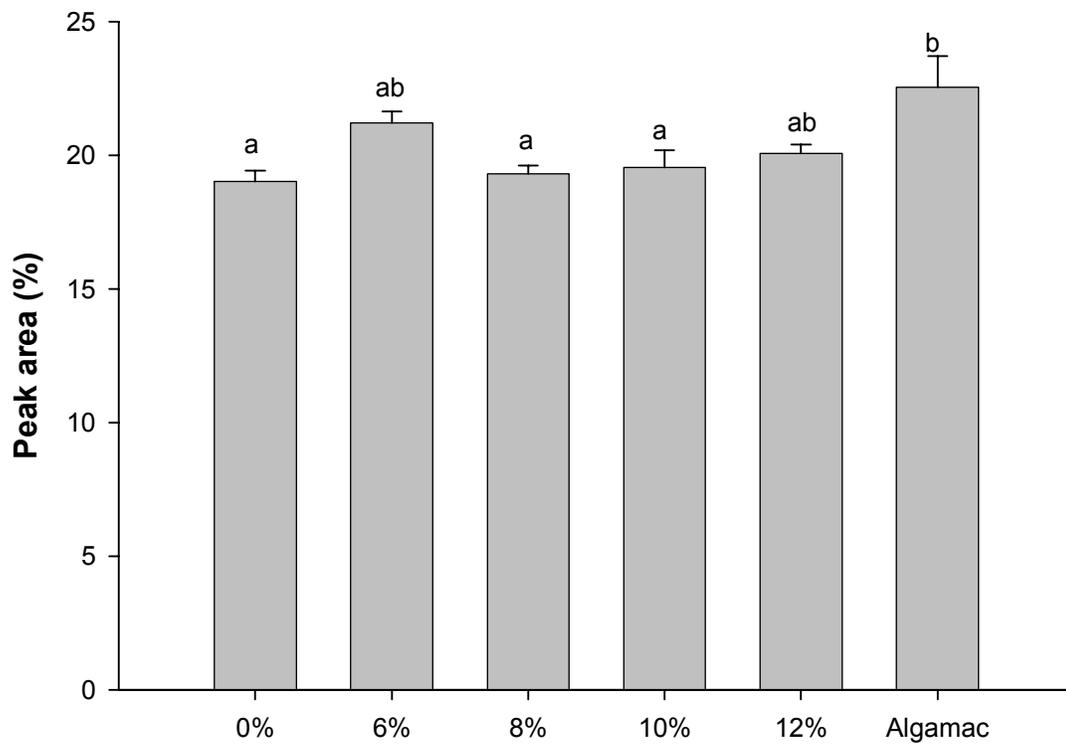


Figure 28. Percentage of linolenic acid, 18:3n-3 ($\bar{X} \pm \text{SEM}$, $N = 3$) detected in black sea bass larvae fed live prey enriched with five different oil emulsions and a commercial diet, Algamac 2000. Each oil emulsion contained 10% DHA and different levels of ARA (0, 6, 8, 10, 12% total lipid). Treatment means not associated with the same letters are significantly different ($P < 0.05$).

20:5n-3 Ecosapentaenoic acid

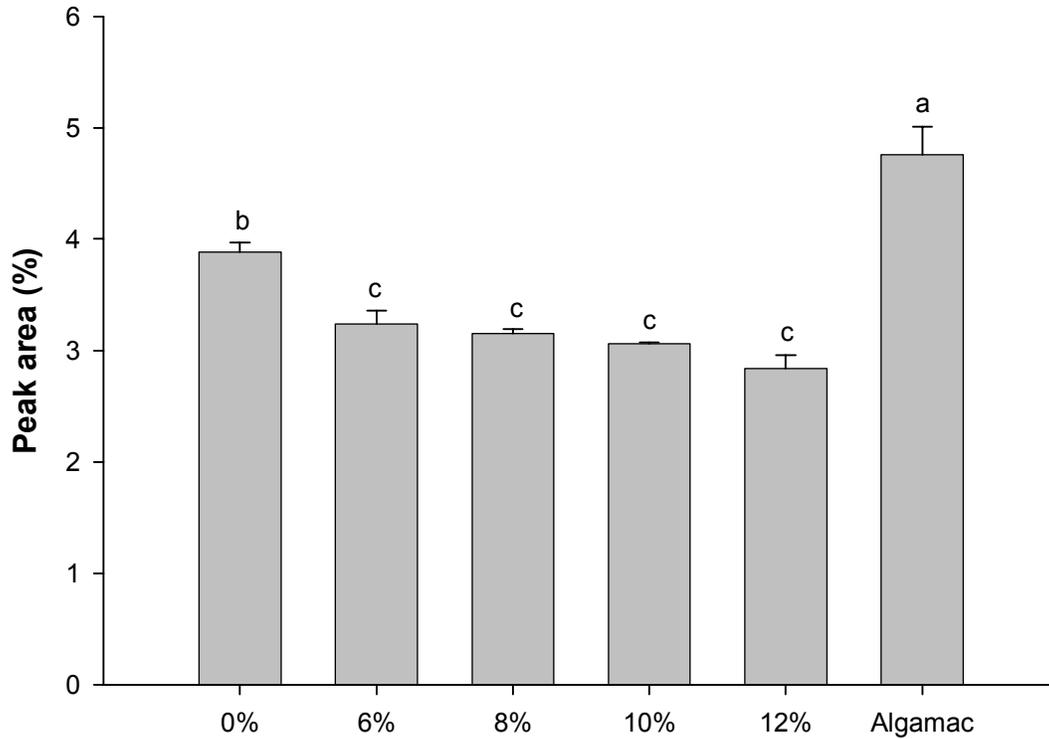


Figure 29. Percentage of ecosapentaenoic acid, 20:5n-3 ($\bar{X} \pm \text{SEM}$, $N = 3$) detected in black sea bass larvae fed live prey enriched with five different oil emulsions and a commercial diet, Algamac 2000. Each oil emulsion contained 10% DHA and different levels of ARA (0, 6, 8, 10, 12% total lipid). Treatment means not associated with the same letters are significantly different ($P < 0.05$).

22:5n-3 (Docosapentaenoic acid)

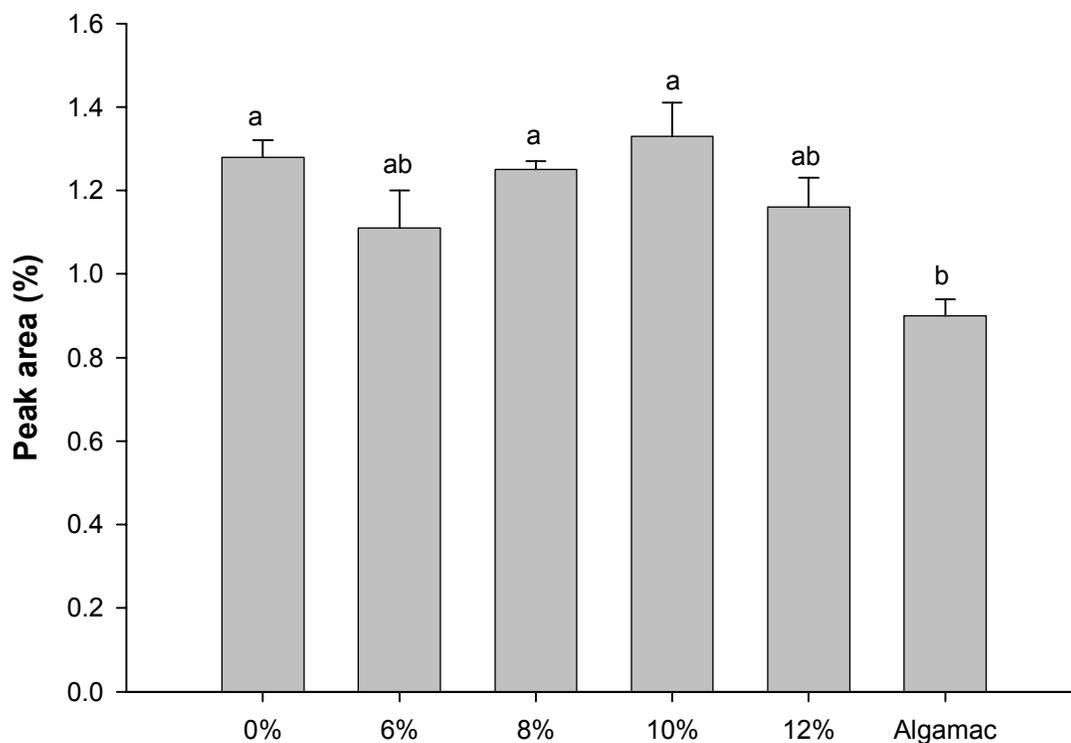


Figure 30. Percentage of docosapentaenoic acid, 22:5n-3 ($\bar{X} \pm \text{SEM}$, $N = 3$) detected in black sea bass larvae fed live prey enriched with five different oil emulsions and a commercial diet, Algamac 2000. Each oil emulsion contained 10% DHA and different levels of ARA (0, 6, 8, 10, 12% total lipid). Treatment means not associated with the same letters are significantly different ($P < 0.05$).

22:6n-3 (Docosahexaenoic acid)

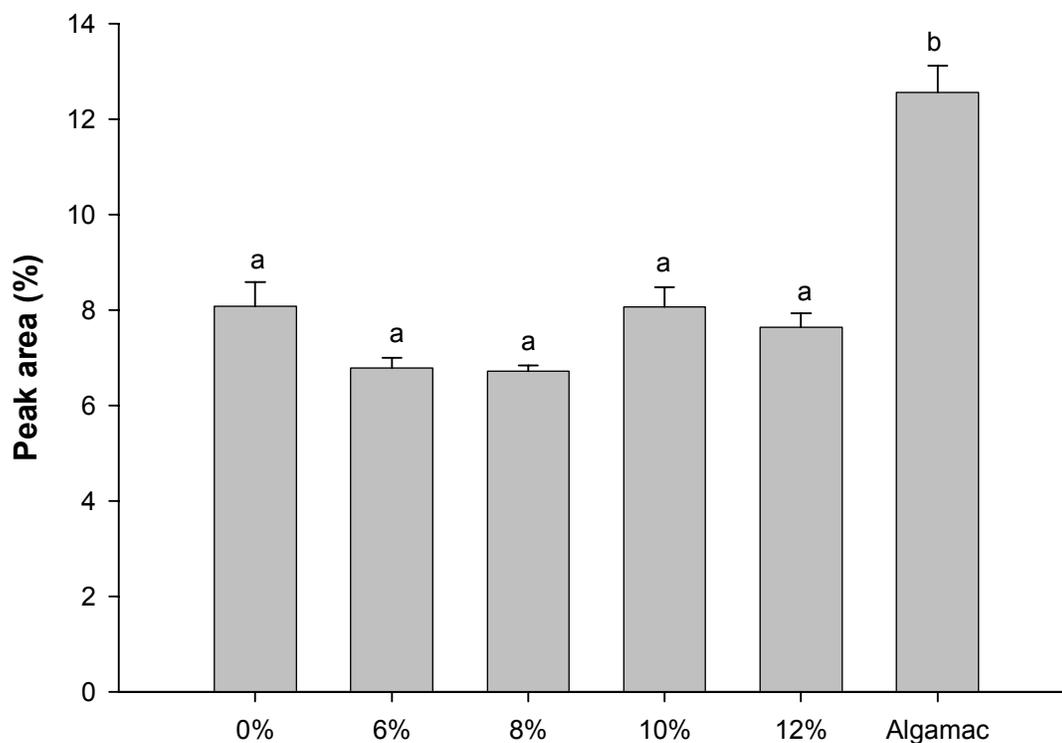


Figure 31. Percentage of docosahexaenoic acid, 22:6n-3 ($\bar{X} \pm \text{SEM}$, $N = 3$) detected in black sea bass larvae fed live prey enriched with five different oil emulsions and a commercial diet, Algamac 2000. Each oil emulsion contained 10% DHA and different levels of ARA (0, 6, 8, 10, 12% total lipid). Treatment means not associated with the same letters are significantly different ($P < 0.05$).

22:6n-3/20:5n-3

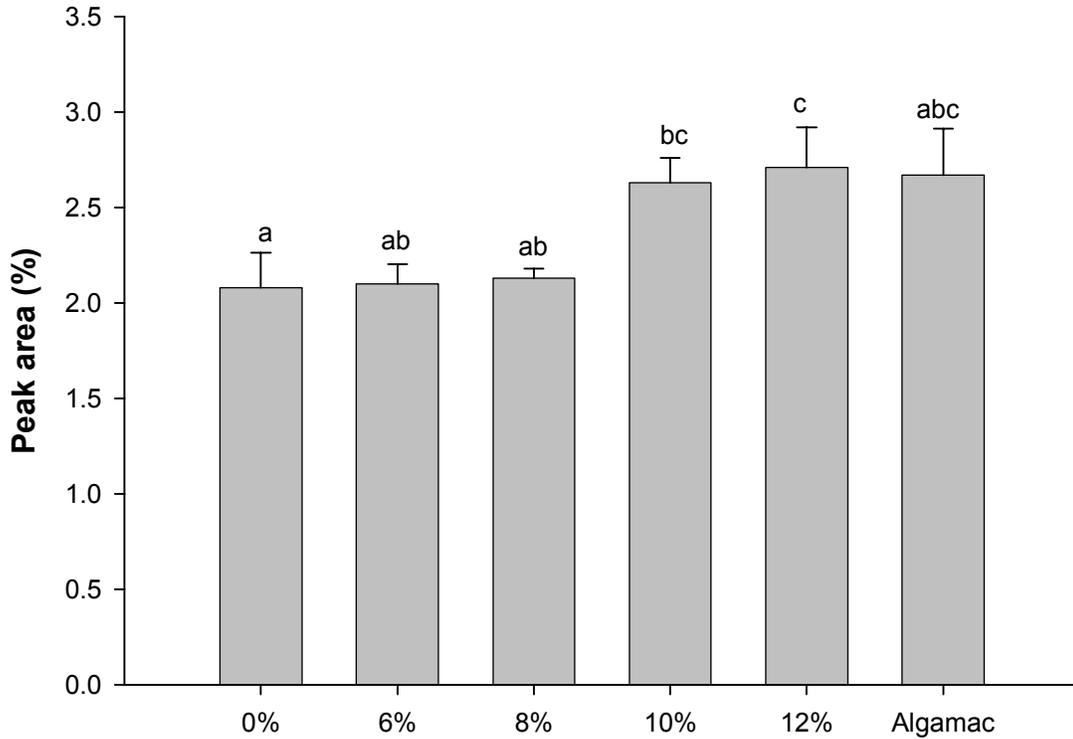


Figure 32. Ratio of 22:6n-3/ 20:5n-3 ($\bar{X} \pm \text{SEM}$, $N = 3$) detected in black sea bass larvae fed live prey enriched with five different oil emulsions and a commercial diet, Algamac 2000. Each oil emulsion contained 10% DHA and different levels of ARA (0, 6, 8, 10, 12% total lipid). Treatment means not associated with the same letters are significantly different ($P < 0.05$).

n-6 Polyunsaturated fatty acid (PUFA)

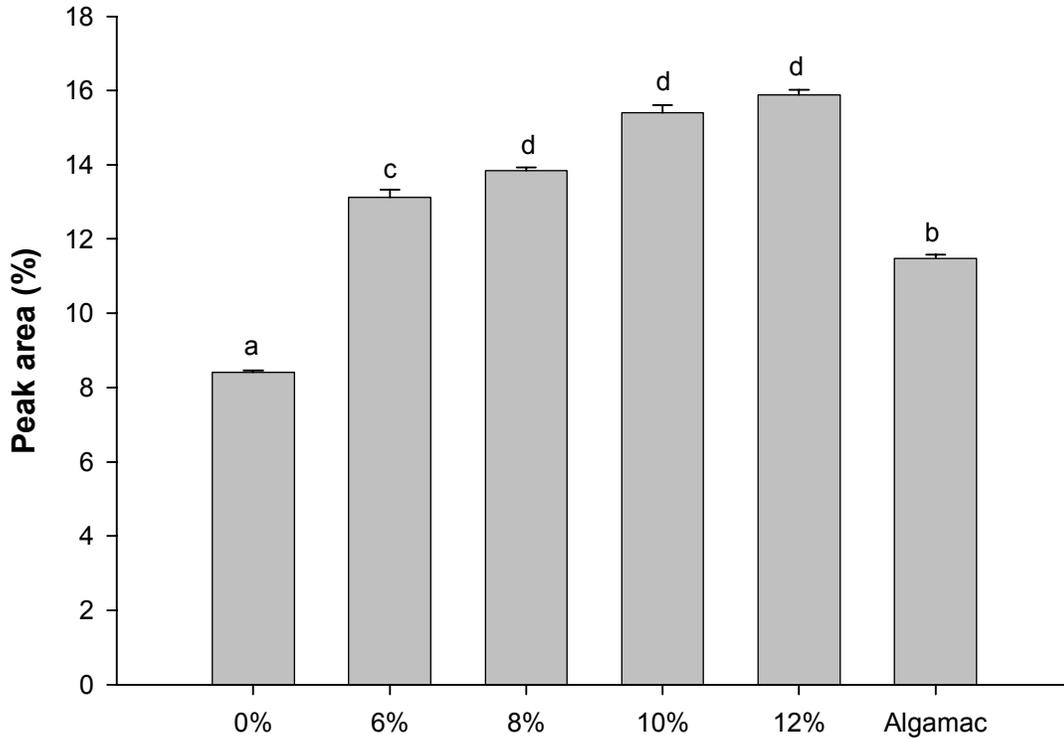


Figure 33. Percentage of n-6 PUFA's ($\bar{X} \pm \text{SEM}$, $N = 3$) detected in black sea bass larvae fed live prey enriched with five different oil emulsions and a commercial diet, Algamac 2000. Each oil emulsion contained 10% DHA and different levels of ARA (0, 6, 8, 10, 12% total lipid). Treatment means not associated with the same letters are significantly different ($P < 0.05$).

18:2n-6 (Linoleic acid)

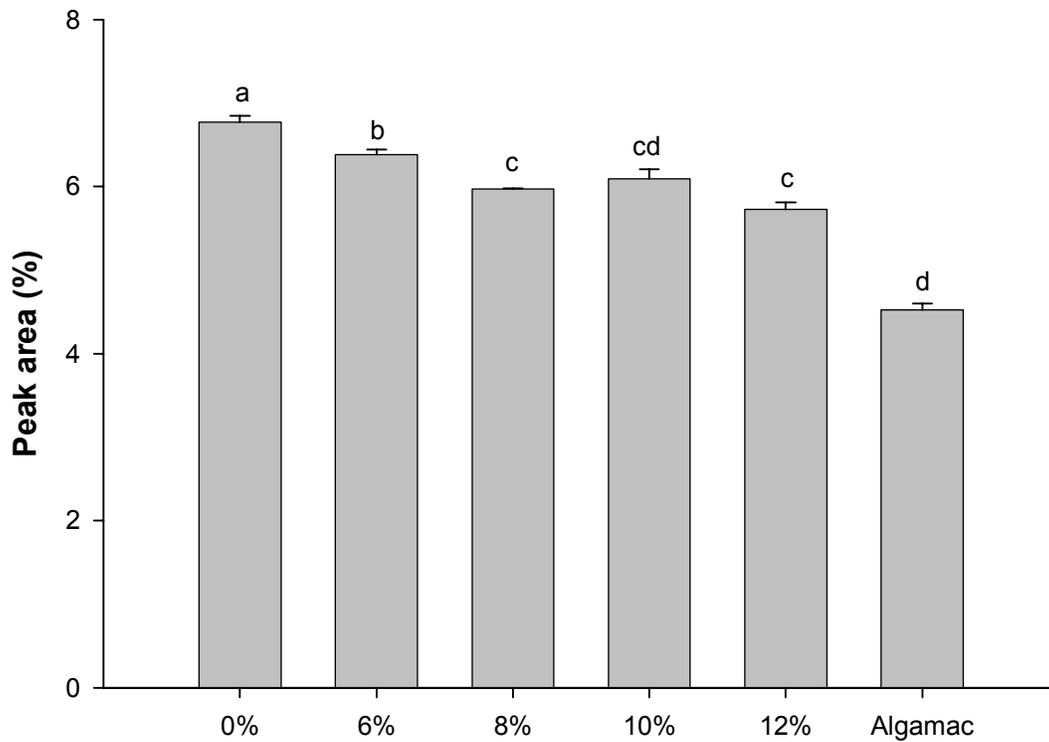


Figure 34. Percentage of linoleic acid, 18:2n-6 PUFA's ($\bar{X} \pm \text{SEM}$, $N = 3$) detected in black sea bass larvae fed live prey enriched with five different oil emulsions and a commercial diet, Algamac 2000. Each oil emulsion contained 10% DHA and different levels of ARA (0, 6, 8, 10, 12% total lipid). Treatment means not associated with the same letters are significantly different ($P < 0.05$).

20:4n-6 (Arachidonic acid)

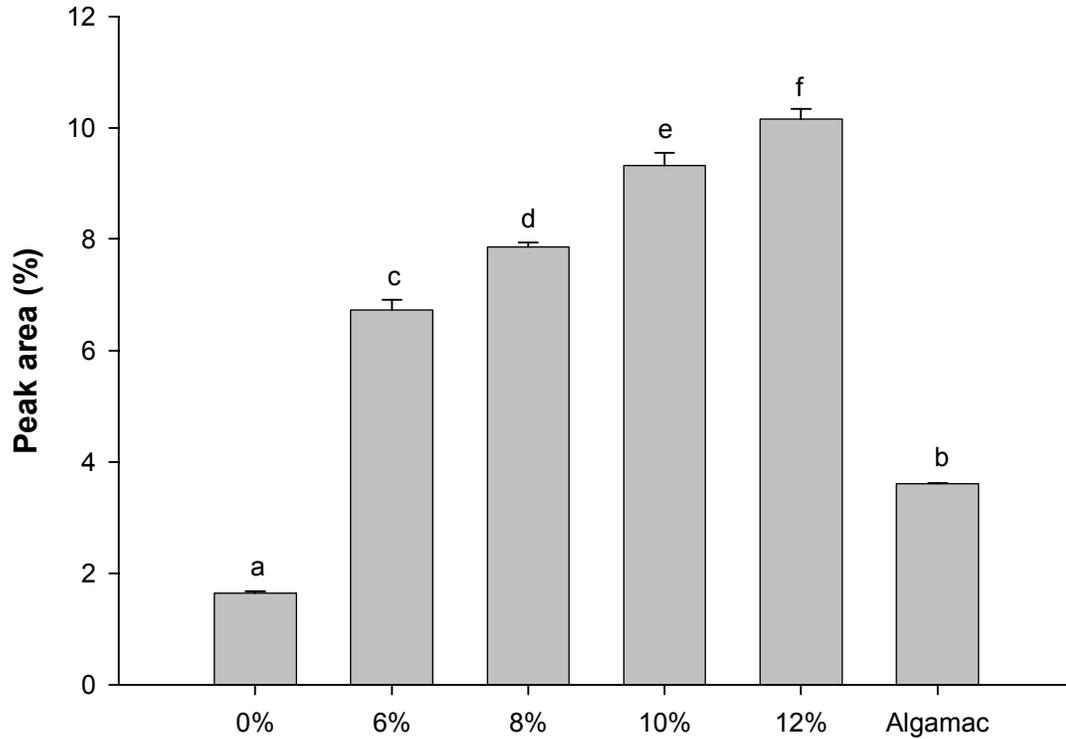


Figure 35. Percentage of arachidonic acid, 20:4n-6 ($\bar{X} \pm \text{SEM}$, $N = 3$) detected in black sea bass larvae fed live prey enriched with five different oil emulsions and a commercial diet, Algamac 2000. Each oil emulsion contained 10% DHA and different levels of ARA (0, 6, 8, 10, 12% total lipid). Treatment means not associated with the same letters are significantly different ($P < 0.05$).

n-3 / n-6 Polyunsaturated fatty acids (PUFA's)

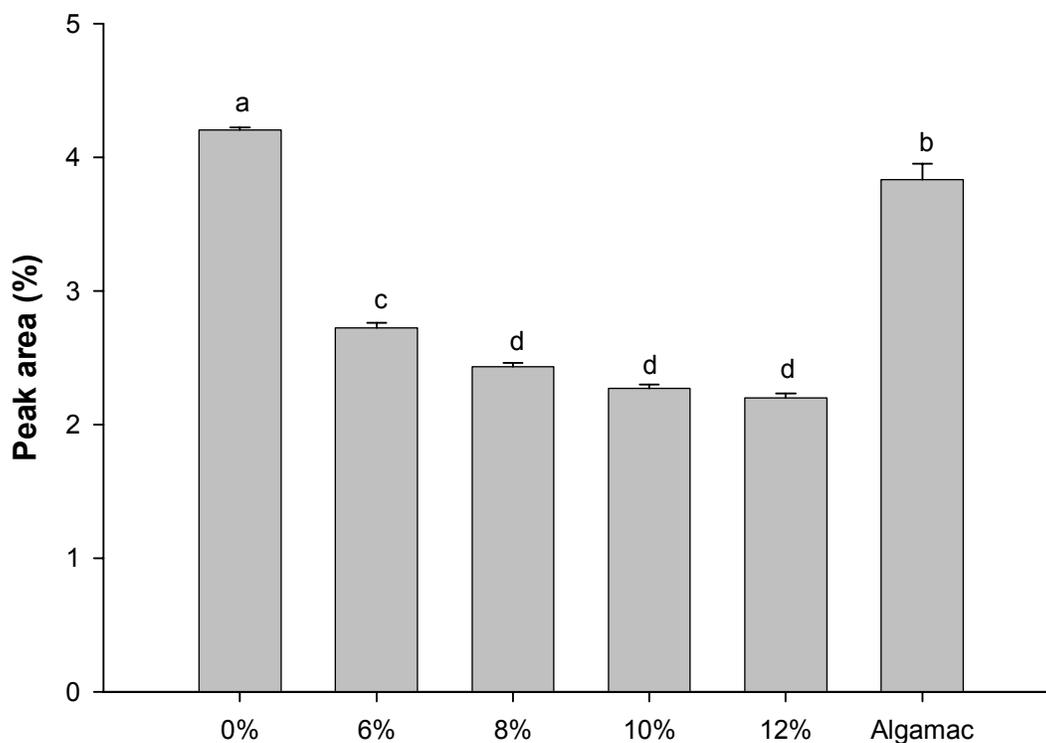


Figure 36. Percentage of n-3/ n-6 polyunsaturated acids, PUFA's ($\bar{X} \pm \text{SEM}$, $N = 3$) detected in black sea bass larvae fed live prey enriched with five different oil emulsions and a commercial diet, Algamac 2000. Each oil emulsion contained 10% DHA and different levels of ARA (0, 6, 8, 10, 12% total lipid). Treatment means not associated with the same letters are significantly different ($P < 0.05$).

22:6n-3/20:4n-6

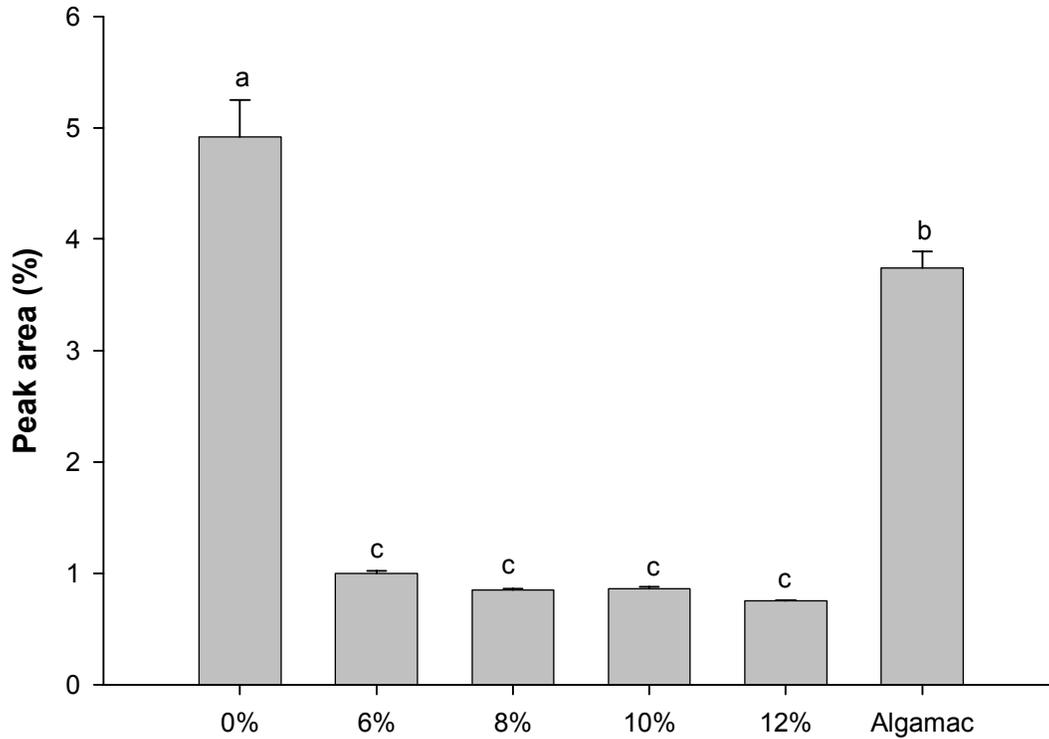


Figure 37. Ratio of 22:6n-3/ 20:4n-6 ($\bar{X} \pm \text{SEM}$, $N = 3$) detected in black sea bass larvae fed live prey enriched with five different oil emulsions and a commercial diet, Algamac 2000. Each oil emulsion contained 10% DHA and different levels of ARA (0, 6, 8, 10, 12% total lipid). Treatment means not associated with the same letters are significantly different ($P < 0.05$).

20:5n-3/20:4n-6

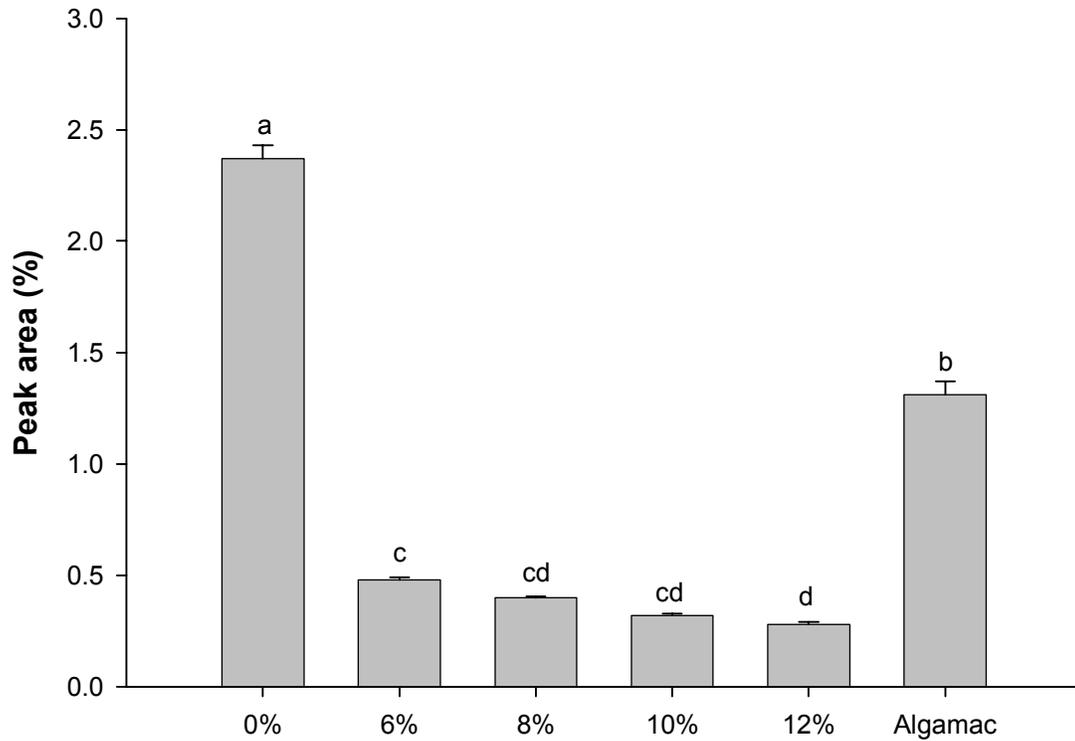


Figure 38. Ratio of 20:5n-3/ 20:4n-6 ($\bar{X} \pm \text{SEM}$, $N = 3$) detected in black sea bass larvae fed live prey enriched with five different oil emulsions and a commercial diet, Algamac 2000. Each oil emulsion contained 10% DHA and different levels of ARA (0, 6, 8, 10, 12% total lipid). Significant ($P < 0.05$) treatment effects were observed. Treatment means not associated with the same letters are significantly different ($P < 0.05$).

Growth

Table 4 shows the notochord length, wet weights and dry weights of BSB larvae fed live prey enriched with Algamac or one of five different oil emulsions containing DHA (10% TFA) and increasing levels of ARA (0, 6, 8, 10, 12% TFA). On d4ph, notochord length averaged 2.94 mm among treatments, with no significant ($P > 0.05$) differences (Fig. 39). Mean notochord length among treatments steadily increased to 4.45, 5.05 and 6.56 mm on d10, d17 and d24ph, respectively, with no significant ($P > 0.05$) treatment differences.

On day 4 post hatching, wet weights averaged 0.11 mg among treatments, with no significant ($P > 0.05$) differences (Fig. 40). Mean wet weights steadily increased to 0.35, 1.13 and 3.60 mg on d10, d17 and d24ph, respectively, with no significant ($P > 0.05$) treatment differences.

Day 4 dry weight data was found to be inaccurate and therefore could not be used for statistical comparison. On day 10 post hatching, dry weights averaged 0.08 mg among treatments, with no significant ($P > 0.05$) differences (Fig. 41). Mean dry weights steadily increased to 0.18 and 0.63 mg on d17 and d24ph, respectively, with no significant ($P > 0.05$) treatment differences.

Table 4. Growth (notochord length, wet weight, dry weight) of black sea bass, *Centropristis striata* larvae fed live prey enriched with different levels of arachidonic acid 20:4n-6 (0 – 12 % total fatty acid) or Algamac 2000. Values are means \pm SEM (n = 5). No significant ($P > 0.05$) treatment differences were observed for any parameter on any sampling date.

Growth		Treatment (% ARA of total lipid and Algamac 2000)					
	Age (dph)	0	6	8	10	12	Algamac
Notochord length (mm)	4	2.88 \pm 0.09	3.03 \pm 0.06	2.92 \pm 0.08	3.03 \pm 0.07	2.98 \pm 0.05	2.85 \pm 0.09
Wet weight (ug)	4	0.14 \pm 0.04	0.12 \pm 0.02	0.11 \pm 0.01	0.16 \pm 0.03	0.08 \pm 0.01	0.10 \pm 0.02
Dry Weight (ug)	4	No data					
Notochord length (mm)	10	4.04 \pm 0.05	3.97 \pm 0.05	3.92 \pm 0.06	3.80 \pm 0.05	3.91 \pm 0.09	4.06 \pm 0.09
Wet weight (ug)	10	0.46 \pm 0.14	0.31 \pm 0.52	0.30 \pm 0.05	0.28 \pm 0.03	0.33 \pm 0.04	0.44 \pm 0.04
Dry Weight (ug)	10	0.08 \pm 0.01	0.07 \pm 0.01	0.07 \pm 0.01	0.07 \pm 0.01	0.07 \pm 0.01	0.09 \pm 0.01
Notochord length (mm)	17	5.16 \pm 0.09	5.18 \pm 0.09	5.21 \pm 0.09	4.94 \pm 0.08	5.0 \pm 0.08	4.83 \pm 0.09
Wet weight (ug)	17	1.17 \pm 0.15	1.24 \pm 0.18	1.23 \pm 0.17	1.09 \pm 0.09	1.02 \pm 0.07	1.05 \pm 0.10
Dry Weight (ug)	17	0.20 \pm 0.02	0.21 \pm 0.03	0.20 \pm 0.03	0.17 \pm 0.01	0.17 \pm 0.02	0.17 \pm 0.02
Notochord length (mm)	24	6.82 \pm 0.16	6.65 \pm 0.14	6.60 \pm 0.12	6.53 \pm 0.11	6.28 \pm 0.11	6.52 \pm 0.10
Wet weight (ug)	24	3.82 \pm 0.86	4.17 \pm 1.01	3.84 \pm 0.64	3.45 \pm 0.48	3.10 \pm 0.58	3.30 \pm 0.44
Dry Weight (ug)	24	0.63 \pm 0.14	0.74 \pm 0.17	0.69 \pm 0.14	0.66 \pm 0.12	0.49 \pm 0.08	0.60 \pm 0.10

Due to small size, accurate measurements of dry weight could not be obtained on d4ph.

Notochord Length

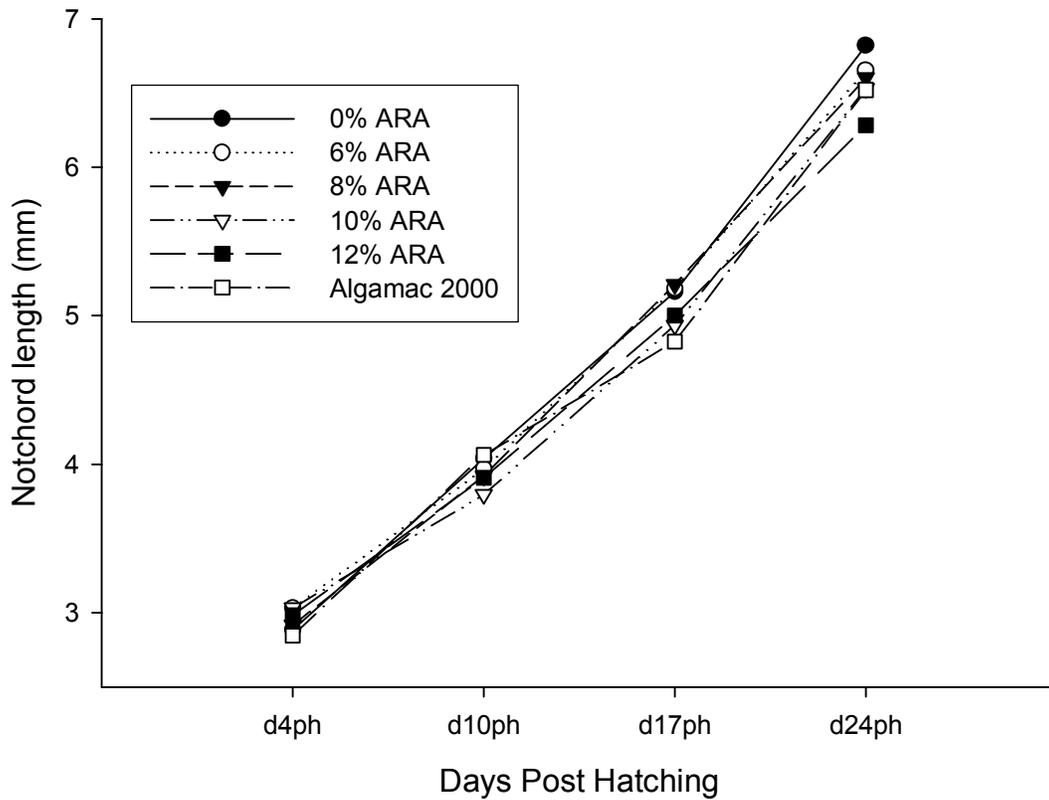


Figure 39. Notochord length of black sea bass (*Centropristis striata*) larvae fed live prey enriched with five different oil emulsions and a commercial diet, Algamac 2000. Plotted symbols represent means (n=5). Each oil emulsion contained 10% DHA and different levels of ARA (0, 6, 8, 10, 12% total lipid). No significant ($P > 0.05$) differences among treatments were detected on any sampling day.

Wet Weight

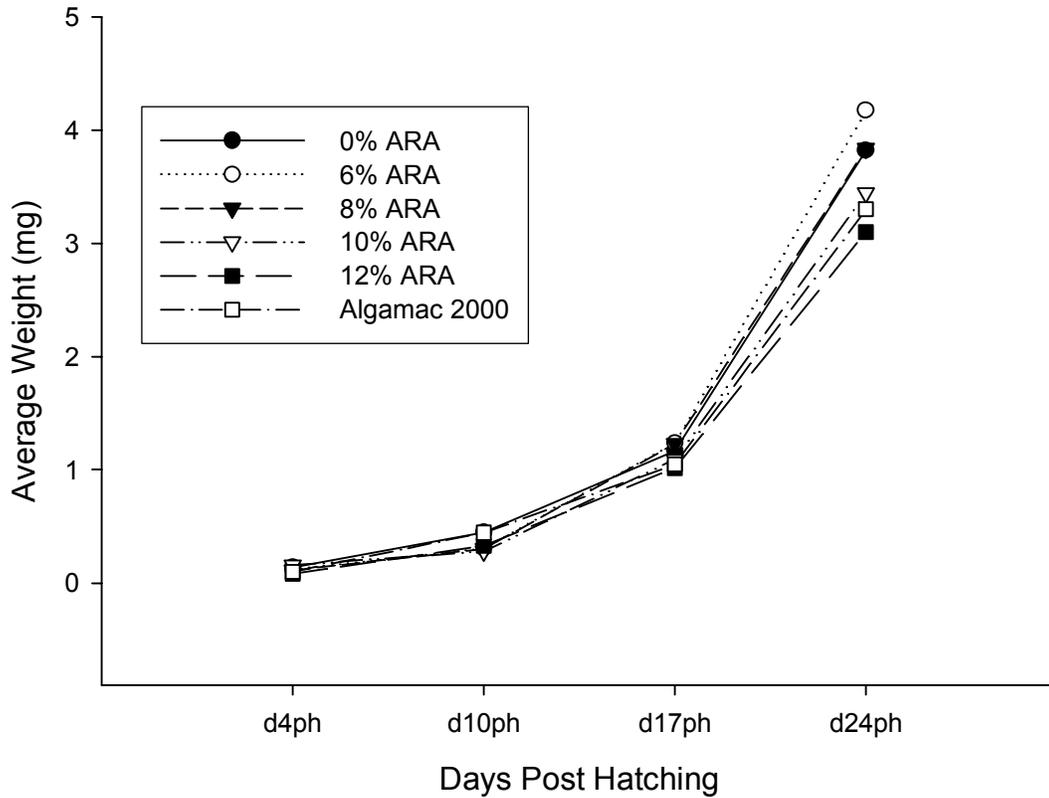


Figure 40. Wet weight of black sea bass (*Centropristis striata*) larvae fed live prey enriched with five different oil emulsions and a commercial diet, Algamac 2000. Plotted symbols represent means ($N=5$). Each oil emulsion contained 10% DHA and different levels of ARA (0, 6, 8, 10, 12% total lipid). No significant ($P > 0.05$) differences among treatments were detected on any sampling date.

Dry Weight

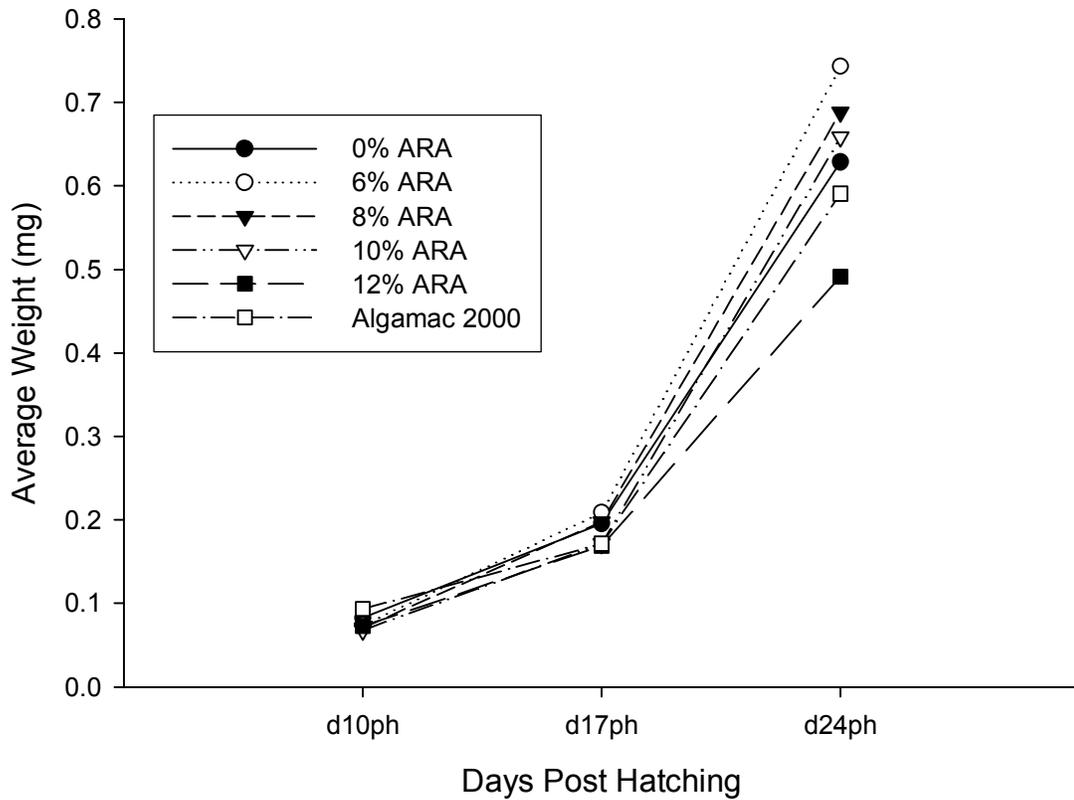


Figure 41. Dry weight of black sea bass (*Centropristis striata*) larvae fed live prey enriched with five different oil emulsions and a commercial diet, Algamac 2000. Plotted symbols represent means ($N=5$). Each oil emulsion contained 10% DHA and different levels of ARA (0, 6, 8, 10, 12% total lipid). No significant ($P > 0.05$) differences among treatments were detected on any sampling date.

Survival

Survival declined sharply by d4ph to a mean of 38.7% among treatments, with no significant ($P > 0.05$) differences (Table 5). After this, survival was relatively stable, with mean values among treatments of 29.5%, 27.3% and 29.3% on d10, d17 and d24ph respectively, with no significant ($P > 0.05$) treatment differences (Fig. 42).

Table 5. Percent Survival of black sea bass *Centropristis striata* larvae fed live prey enriched with different levels of arachidonic acid 20:4n-6 (0 – 12 % total fatty acid) or Algamac 2000. Values are means \pm SEM (n = 5).

Survival (%)						
Age (dph)	Treatment (% ARA of total lipid and Algamac 2000)					
	0	6	8	10	12	Algamac
4	31.7 \pm 0.12	25.9 \pm 0.04	32.2 \pm 0.08	56.9 \pm 0.12	43.8 \pm 0.07	41.9 \pm 0.10
10	23.0 \pm 0.06	24.9 \pm 0.06	27.1 \pm 0.05	36.9 \pm 0.05	33.4 \pm 0.05	31.8 \pm 0.08
17	18.2 \pm 0.05	27.2 \pm 0.06	27.3 \pm 0.06	31.7 \pm 0.06	25.0 \pm 0.03	34.3 \pm 0.11
24	24.3 \pm 0.08	28.0 \pm 0.05	30.1 \pm 0.05	32.7 \pm 0.08	30.7 \pm 0.03	30.2 \pm 0.08

Mean values in a row followed by different letters are significantly ($P < 0.05$, ANOVA) different.

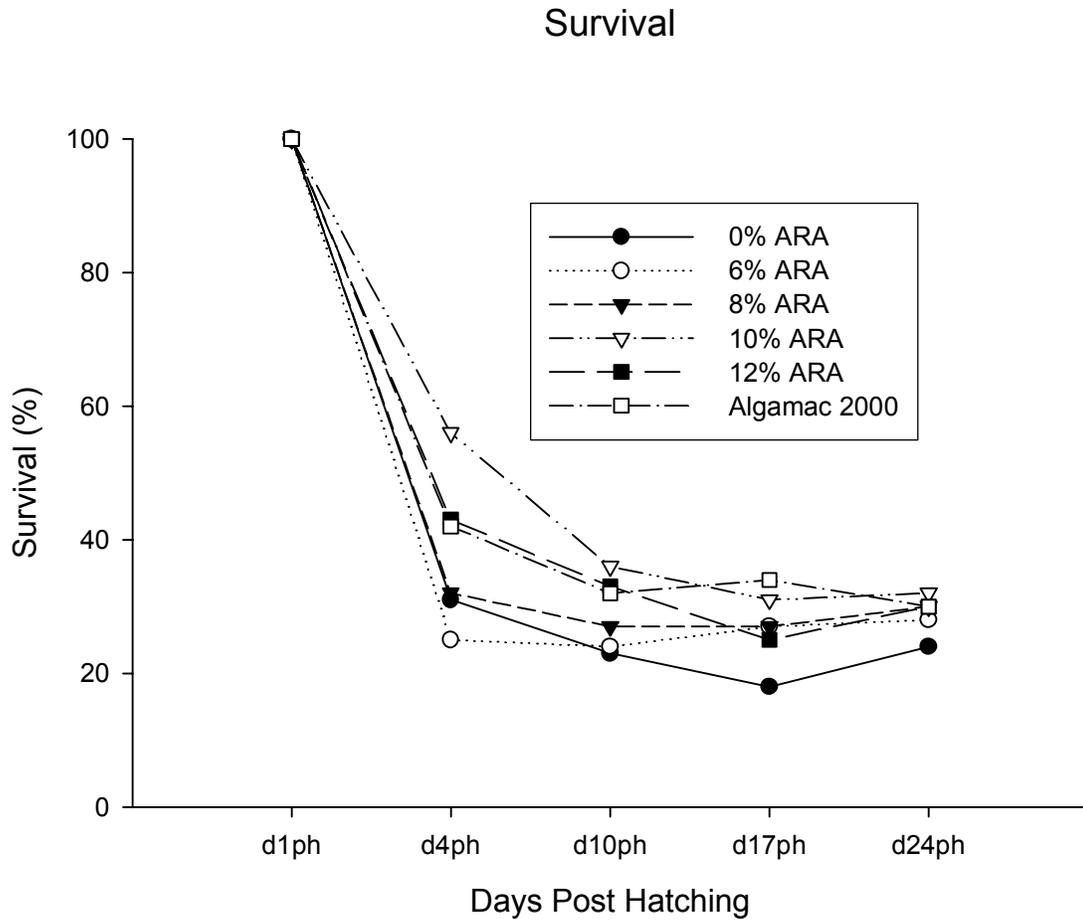


Figure 42. Survival of black sea bass (*Centropristis striata*) larvae fed live prey enriched with five different oil emulsions and a commercial diet, Algamac 2000. Plotted symbols represent means ($N=5$). Each oil emulsion contained 10% DHA and different levels of ARA (0, 6, 8, 10, 12% total lipid). No significant ($P > 0.05$) differences among treatments were detected on any sampling date.

Hypersaline Stress Evaluation

ST50 for d24ph black sea bass larvae subjected to a hypersaline challenge of 55 g/L ranged from 27.1 to 31.8 min among treatments, with no significant ($P > 0.05$) differences (Table 6). MST for d24ph black sea bass larvae subjected to a hypersaline challenge of 55 g/L ranged from 32.5 to 35.5 min among treatments, with no significant ($P > 0.05$) differences (Table 6).

Table 6. Hypersaline stress resistance of d24ph black sea bass larvae, *Centropristis striata* fed live prey enriched with different levels of arachidonic acid 20:4n-6 (0 – 12 % total fatty acid) or Algamac 2000. Larvae were subjected to a hypersaline stress test of 55 ppt. ST50 and MST data are reported as mean \pm SEM, n = 5. No significant ($P > 0.05$) treatment differences were observed.

	Treatment (% ARA of total lipid and Algamac 2000)					
	0	6	8	10	12	Algamac
ST50	31.82 \pm 1.88	29.32 \pm 2.77	27.14 \pm 4.28	29.06 \pm 3.29	31.61 \pm 3.46	28.95 \pm 3.76
MST	35.22 \pm 1.86	32.98 \pm 2.79	33.06 \pm 5.67	32.52 \pm 3.28	35.51 \pm 3.61	32.83 \pm 3.85

Relative Na⁺ K⁺ ATPase Expression

The relative mRNA expression of Na⁺ K⁺ ATPase (NKA) in d24ph black sea bass larvae before exposure to a sub-lethal salinity of 43 ppt ranged from 0.27 to 0.47 among treatments with no significant ($P > 0.05$) differences (Table 7). The relative mRNA expression of NKA in d24ph black sea bass larvae after 24 h of exposure to a sub-lethal salinity of 43 ppt ranged from 0.64 to 0.92 among treatments with no significant ($P > 0.05$) differences (Fig. 43). Larvae fed dietary ARA of 6-12% had significantly ($P < 0.05$) higher levels of NKA after 24 h than at 0 h. In contrast, there was no significant ($P > 0.05$) increase in the level of NKA detected in the 0% ARA or ALG treatments after 24 h.

mRNA Expression

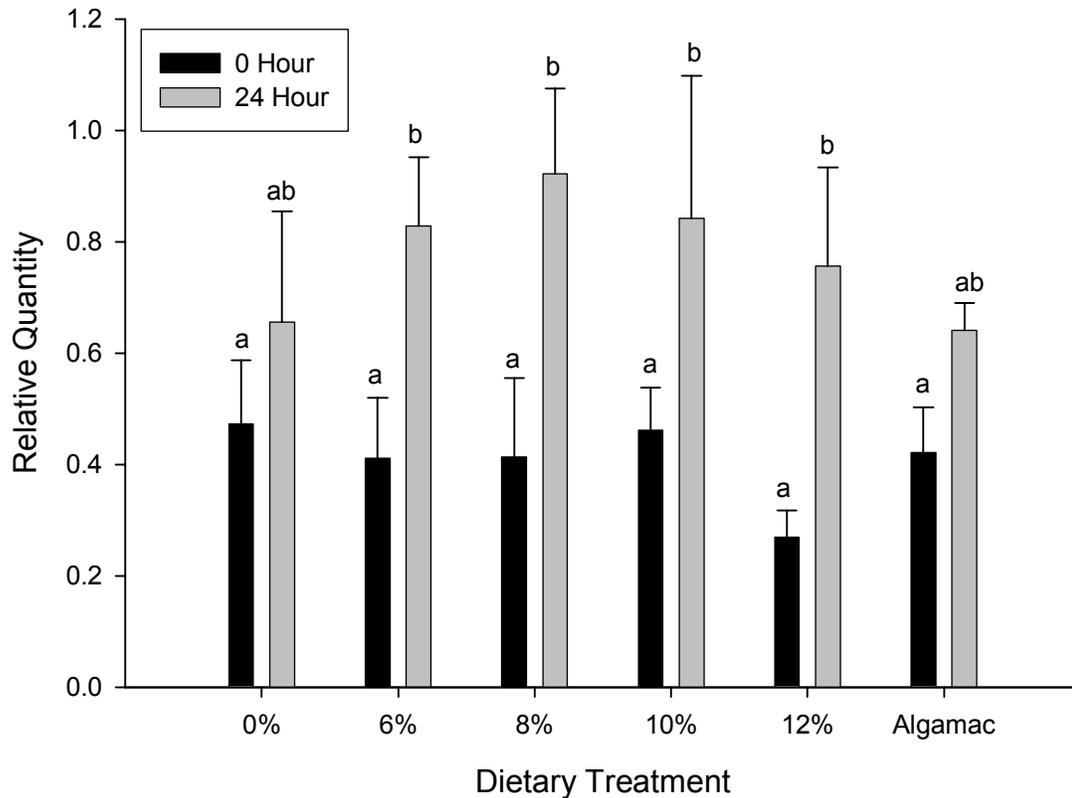


Figure 43. Relative expression of Na⁺ K⁺ ATPase in black seabass (*Centropristis striata*) larvae fed live prey enriched with different levels of arachidonic acid 20:4n-6 (0 – 12% total fatty acid) or Algamac 2000. Values are means ± SEM, (N = 3). Larvae were subjected to an increase in salinity of 8 ppt (from 35 to 43 ppt), sampled before transfer (0 h, dark bars) and at 24 h (light bars) post transfer. For each time period (i.e. comparisons among dietary treatments) and for each treatment (i.e. 0 vs 24 h), bars not associated with the same letter are significantly (P < 0.05) different.

DISCUSSION

Fatty Acid Composition of Enriched Rotifers

Although rotifers are not a natural food source for marine finfish they are commonly used in larval nutrition studies because they are relatively easy and reliable to culture in high densities and can be manipulated to contain specified levels of essential fatty acids (Estevez et al., 1999; Koven et al., 2001; Willey et al., 2003; Bransden et al., 2004; Faulk et al., 2005a). Rotifer fatty acid profiles were significantly affected by the commercial diet Algamac 2000 (ALG) and the experimental ARA emulsions. The total lipid of the rotifers did not differ among treatments. Because of the high levels of myristic acid (16.3% TFA) and palmitic acid (42.2% TFA) in the commercial diet ALG, both of these saturated fatty acids were retained in rotifers at significantly higher levels in this treatment compared to the ARA diets. There was no difference in these saturates among the ARA diets.

The olive oil in the ARA emulsions, which is high in oleic acid, caused the level of MUFA's to decrease with increasing ARA. The lowest levels of oleic acid were found in the commercial diet ALG, which contained no olive oil. Levels of oleic acid in ALG were nearly 1/3 of the mean amount found in the highest ARA treatment which contained the lowest concentration of olive oil. Bransden et al. (2004) also found that increasing dietary HUFA decreased total oleic acid in rotifers following enrichment.

As expected, a higher level of total n-3 PUFA was found in the ALG treatment than in the ARA treatments. This is primarily attributed to the high level of DHA in ALG (27% TFA) (Aquafauna Bio-Marine, Hawthorne, CA, USA) compared to the

experimental emulsions that had only 10% DHA. Other studies have shown ALG to be a very good booster of DHA in rotifers. Faulk et al. (2005b) enriched rotifers with ALG for 16 h and attained DHA levels as high as 24.9% TFA. In another study, Harel et al. (2002) enriched rotifers for 8 h with ALG and boosted DHA to a level of 23.4% TFA. DHA levels in rotifers remained the same in all the ARA treatments (mean = 10.2%) and differed only from the ALG treatment (19.8%). EPA was similar (7.94% TFA), regardless of dietary treatment.

Of the n-6 PUFA's, rotifers retained ARA efficiently, with post-enrichment concentrations approaching emulsions levels. ALG was the only treatment yielding docosapentaenoic acid (DPA_n-6) in rotifers at a concentration of 6.05% TFA, which is in agreement with other studies using this commercial diet (Koven et al., 2001; Bransden et al., 2004). This was expected since DPA_n-6 is present in ALG at a level of 10.6% TFA (Aquafauna Bio-Marine, Hawthorne, CA, USA).

Mammals can retroconvert DPA_n-6 to ARA, and Barclay et al. (1996) suggested that rotifers may be capable of this conversion. In this study, however, no retroconversion was evident, since there were no differences in ARA levels between ALG and the 0% ARA treatments. The rotifers were raised on *N. oculata*, which naturally contains ARA (3.94% TFA), and this caused the levels of ARA in all of our treatments to be slightly higher than prescribed (Reed Mariculture, Campbell, CA, USA). For instance, the 0% diet had 1.62% ARA present and the ALG treatment had 2.88% ARA. ARA concentrations in these diets were not significantly different, but they were significantly lower than in the 6-12% ARA treatments (range = 8.27-14.3% ARA as TFA).

Fatty Acid Composition of Enriched Artemia

Like rotifers, Artemia have been used extensively in commercial larval culture of marine finfish because they are reliable to culture and relatively easy to enrich with various nutritional components. They are a larger live prey organism than the rotifers and can be used as an intermediate feed between rotifers and microparticulate diets (Estevez et al., 1999; Harel et al., 2001; Copeman et al., 2002; Koven et al., 2003; Willey et al., 2003; Bransden et al., 2004; Faulk et al., 2005b). In this study, no differences among treatments were found in total lipid or saturates in Artemia. As observed in rotifers, MUFA's decreased as ARA increased due to the varying concentrations of oleic acid in our enrichments. These findings are in agreement with a study by Copeman et al. (2002) in which the highest levels of MUFAs were in the olive oil diet they used as a control.

An interesting finding was the total n-3 PUFA was lowest in HUFA – rich (highly unsaturated fatty acid) commercial diet, ALG at 24% compared to 34.9% found in the experimental ARA diets. Linolenic acid (18:3n-3) was one of the largest contributors to n-3 PUFA in the ARA diets. ALG contributed almost 2.5 times the amount of DHA as the experimental emulsions, but DHA in Artemia enriched with the ALG treatment was only 10.9% compared to 6.09% in the ARA diets. This low DHA in the ALG treatment can be attributed to the retroconversion of DHA to EPA by the Artemia (Navarro et al., 1999). The difficulty of enriching Artemia with high DHA using ALG was previously reported by Harel et al. (2002) who found that rotifers retained three times more DHA

(23.4% TFA as DHA) than Artemia (7.4% TFA as DHA). Koven et al. (2001) observed a marked decrease in the DHA/ARA ratio in Artemia enriched with ALG (4.6) compared to rotifers fed ALG (10.1). Because of the selective catabolism of DHA it is difficult to bioencapsulate Artemia with very high levels of DHA (Navarro et al., 1999; Sorgeloos et al., 2001). By the time Artemia reach Instar II (6-8 h after hatching), endogenous reserves are used up and the nauplii are one and half times larger (Sorgeloos et al., 2001). DHA is a good source of energy and therefore it is selectively catabolized into EPA (Sorgeloos et al., 2001). It is essential to ensure that the Artemia are being enriched as they reach instar II when they begin feeding (Sorgeloos et al., 2001). It is possible that our 18-h Artemia enrichment period coincided with the early part of the first naupliar stage, decreasing the efficiency of bioencapsulation. Other studies have achieved higher levels of DHA in Artemia by enriching for 24 and 32 h to boost the enrichment level as many as 2 to 3 times (Sorgeloos et al., 2001 and Faulk et al., 2005). Hence, a higher level of DHA in Artemia could likely be achieved by enriching for 24 h or more.

EPA in Artemia was twice as high in the ALG (3.6%) than in the ARA treatments (1.76%). In contrast, levels of EPA in the rotifers (7.94%) remained relatively stable among all of the treatments, suggesting that Artemia catabolized DHA to EPA. Due to this conversion of DHA to EPA, the ratio of DHA/EPA (mean = 3.4) in Artemia remained relatively constant among all treatments, even though there was more DHA available in ALG than in the ARA treatments. The ratio exceeded the recommended minimum of 2:1 (DHA/EPA) for marine finfish larvae (Lavens et al., 1995, pp. 373-397; Lee and Ostrowski, 2001; Kanazawa, 2003).

The n-6 PUFA's in Artemia increased with increasing dietary ARA. Enriched with the same emulsions as the rotifers, Artemia fed 0% ARA contained no detectable ARA further supporting N. oculata as the origin of the ARA found in rotifers. Artemia fed ALG contained 1.74% ARA as total lipid even though this commercial diet is devoid of ARA (Aquafauna Bio-Marine, Hawthorne, CA, USA). This was probably related to retroconversion of DPAn-6 (which is found only in ALG) to ARA, as previously reported in Artemia (Barclay and Zeller, 1996; Navarro et al., 1999; Faulk et al., 2005a). On the other hand, Artemia do not typically have DPAn-6, and there was no DPAn-6 in the 0-12% ARA treatments, or ARA detected in the 0% ARA treatment; therefore, the DPAn-6 in ALG was indeed retroconverted to ARA in Artemia.

Willey et al. (2003) found that rotifers incorporated both DHA and ARA more efficiently during enrichment than Artemia. A higher level of HUFA incorporation in rotifers is primarily attributed to the Artemia's high rate of metabolism during the enrichment period. After enrichment, storage at temperatures below 10° C reduces metabolism in Artemia and enables the retention of HUFA's for longer periods of time (Sorgeloos et al., 2001).

Fatty Acid Composition of d24ph Larvae

As previously observed in striped trumpeter (Bransden et al., 2004), yellowtail flounder Limanda ferruginea (Copeman et al., 2002), turbot Scophthalmus maximus (Estevez et al., 1999), yellowtail snapper Ocyurus chrysurus (Faulk et al., 2005b), and Japanese flounder Paralichthyes olivaceus (Furuita et al., 1998), there was a strong

relationship between whole body tissue fatty acids and dietary fatty acids in larval black sea bass. Black sea bass larvae showed no differences among dietary treatments in total lipids or in saturated fatty acid profiles. Since live feeds were enriched to contain decreasing levels of olive oil as ARA levels increased, larval MUFA decreased from 31.2- 22.4% (TFA) as dietary ARA increased from 0 – 12% TFA, while larvae fed ALG contained only 18.1% TFA as MUFA. Oleic acid ranged from 26.3-18.3% in the ARA treatments and was only 13.3% in ALG. Bransden et al. (2004) fed striped trumpeter larvae ARA – rich oil (Vevodar®, VVO, DSM Food Specialties, Netherlands) in a range of 0 to 36% supplemented with olive, canola and soy bean oils. As was observed in black sea bass, striped trumpeter showed decreasing tissue oleic acid concentrations with increasing ARA concentrations

The total amount of n-3 PUFA's in black sea bass larvae differed significantly between the experimental diets and ALG. In the ALG treatment (43.9% TFA as n-3 PUFA's), total n-3 PUFA's were 9% higher than the ARA treatments (mean of 35% TFA as n-3PUFA's). DHA accounted for approximately 5% of this difference between the ARA (7.5% TFA) and ALG (12.6%) fed larvae, while the combined value of the other n-3 PUFA's detected (e.g. 18:3n-3, 18:4n-3, 20:5n-3 and 22:5n-3) in ALG fed larvae accounted for the remaining difference.

Gilthead sea bream larvae were found to preferentially accumulate ARA rather than EPA in the presence of increasing dietary ARA, suggesting a competitive interaction between ARA and EPA incorporation in tissue (Bessonart et al., 1999). In turbot, varying ARA within an intermediate range produced similar levels of EPA, but produced different EPA levels at extremely low or high ARA levels (Estevez et al., 1999).

Brandsen et al. (2004) reported decreasing tissue EPA in striped trumpeter with increasing ARA. In larval black sea bass, a trend of decreasing EPA/ARA ratios in tissue was observed with increasing ARA, supporting the idea that dietary ARA affects tissue EPA. From our data it appears that the black sea bass preferentially incorporate ARA rather than EPA from their diets, similar to yellowtail snapper (Faulk et al., 2005b) and striped bass Morone saxatilis (Harel et al., 2001) where increasing dietary ARA caused decreasing EPA in larval tissues.

Total n-6 PUFA's found in black sea bass larvae was significantly affected by our experimental ARA treatments as well as by the commercial diet ALG. In general, increasing dietary ARA, caused an increase in total n-6 PUFA's. Larval tissue ARA closely reflected dietary treatments and were significantly different from one another. The ALG treatment had a level of ARA (3.61% TFA) intermediate to the 0% and 6% ARA treatments (1.64 – 6.73 %TFA). Interestingly, in the 0% ARA treatment, larvae averaged 1.64% (TFA as ARA) having been fed both live rotifers (1.62% TFA as ARA) and Artemia (0% TFA as ARA). This ARA originated from the Nannochloropsis fed rotifers as discussed earlier.

Larvae in all treatments grew at the same rate and showed similar mortalities throughout the study suggesting that even small amounts of dietary ARA supported growth and survival to the same extent as high ARA (12% ARA as TFA). Essentially, larvae fed ALG were not fed more ARA than larvae in the 0% ARA treatment until they were fed Artemia which are able to retroconvert DPAn-6 to ARA, which consequently raised the levels of ARA in larvae significantly (Navarro et al., 1999). It is possible that larval black sea bass are capable of this conversion as found earlier in gilthead sea bream

larvae (Koven et al., 2001). Faulk et al. (2005a) found that cobia fed ALG supplemented with ARA by 10 and 20% (Aquagrow, Advanced Bionutrition, Columbia, Maryland, USA) showed no differences in total ARA levels in larval tissues compared to ALG alone. They suggested that cobia may be capable of retroconverting DPAn-6 obtained from ALG treatments, which masked the effects of an increase in dietary ARA and causing similar ARA levels within larval tissues.

Growth

In this study, no differences in growth (notochord length, wet weight and dry weight) of black sea bass larvae fed dietary ARA ranging from 0 to 12% TFA were observed. However in all treatments, average notochord length (2.94 – 6.56 mm) and wet weight (0.11 – 3.60 mg) increased from d4 to d24ph. Although dry weight data was not available for d4ph, dry weight increased from 0.08 mg on d10ph to 0.63 mg by d24ph. These results are in agreement with studies on larval gilthead sea bream (Bessonart et al., 1999), turbot (Estevez et al., 1999), yellowtail snapper (Faulk et al., 2005b) and cobia (Faulk et al., 2005a) where variations in dietary ARA (dry weight or in % TFA) within a range of 0 to 20% had no effect on growth. In gilthead sea bream, no significant differences in growth were observed in larvae (d31ph) fed a microparticulate diet varying in ARA (0.1 – 1.8% ARA as DW). The ratio of DHA/EPA was 1.8 for all ARA treatments (Bessonart et al., 1999). Larval turbot subjected to an increase in dietary ARA from 0.9 to 12% (ARA as DW) showed no differences in growth (Estevez et al., 1999). Larval yellowtail snapper fed commercial diets supplemented with ARA ranging from

1.8 to 5.4% TFA showed no differences in growth (Faulk et al., 2005b). Cobia fed ALG supplemented with ARA at levels of 0, 10 and 20% (replacing ALG) did not show differences in growth or in ARA levels in larval tissue (Faulk et al., 2005a).

In contrast to the black sea bass, gilthead sea bream, turbot, yellowtail snapper, and cobia, striped bass Morone saxatilis (Harel et al., 2001) and yellowtail flounder (Copeman et al., 2002) fed increasing dietary ARA grew faster than those fed diets deficient in ARA. Striped bass fed diets ranging from 4.7 to 19.6 mg/g (ARA as DW) were found to have optimum growth at 14.5 mg/g (ARA as DW) (Harel et al., 2001). In larval striped trumpeter Latris lineata, no differences growth were observed using an ARA – rich oil (Vevodar®) in the range of 0 to 36% TFA (Bransden et al., 2004). However, the same ARA diets produced significantly smaller larvae compared to larvae fed the commercial diet ALG, possibly due to other nutrients present in ALG such as vitamins, minerals and proteins not present in experimental diets (Bransden et al., 2004).

Since differences in growth of larval black sea bass caused by ARA were not observed by day 24 post – hatching it is possible that ARA's role in growth may be observed during the metamorphic stage after weaning from live feeds or under handling and environmental stresses common to hatcheries. ARA used as the only EFA produced better growth in juvenile turbot than combinations of DHA/ARA or DHA alone, suggesting the effects of ARA on growth may be more clearly manifested in later life stages of the turbot (Castel et al., 1994). Summer flounder Paralichthys dentatus fed enriched rotifers with increasing ARA ranging from 0 to 12% TFA showed no differences in growth by 18 days post – hatching. However in a second trial where larvae were fed ARA – enriched rotifers (0, 6% ARA as TFA) for 23 days after hatching and

then, fed un-enriched Artemia through d45ph, the high ARA (6%) treatment showed better growth (Willey et al., 2003). Gilthead sea bream (premetamorphic larvae) fed rotifers enriched with ARA ranging from 1.14 to 3.86 mg/g showed no differences in growth by d18ph (Koven et al., 2003). After metamorphosis, however, larvae fed dietary ARA ranging from 0.59 to 5.86 mg/g (DW as ARA) showed better growth at the lowest levels of ARA on day d42ph after being subjected to an acute stress, whereas no differences were observed between larvae fed the same diets and subjected to a chronic stress.

Available data indicate that DHA may exert a more important effect than ARA during early larval development in some marine finfish species. In larval yellowtail flounder, diets high in ARA (9%) produced better growth than a control diet deficient in ARA (0%). However the 9% ARA treatment did not grow as well as larvae fed diets high in DHA and deficient in ARA further supporting the importance of DHA's role in larval growth over ARA (Copeman et al., 2002). Rezek et al. (2005) showed that black sea bass larvae fed diets deficient in DHA were smaller than larvae fed 10% DHA, but no differences in growth due to increasing ARA within a range of 0 to 6% TFA were observed. In larval yellowtail flounder, diets high in ARA (9%) produced better growth than a control diet deficient in ARA (0%). However the 9% ARA treatment did not grow as well as larvae fed diets high in DHA and deficient in ARA further supporting the importance of DHA's role in larval growth over ARA (Copeman et al., 2002).

Based on the results of this study, the role of ARA in growth in larval black sea bass is not clear. Fish showed similar growth in the experimental and commercial diets, probably related to the high level of DHA in these diets. Additional research is needed to

determine an optimum dietary ARA level for growth in larval black sea bass as well as levels higher than optimum which produce adverse effects on growth. These effects may become manifested during the post-metamorphic stages. Longer term experiments may reveal ARA's effect on growth.

Survival

In this study, black sea bass larvae were stocked on d1ph at an initial density of 100 ind./L and survival in all treatments decreased considerably by d4ph (mean = 38.7%). Between d10ph and metamorphosis (d24ph), survival remained relatively constant among treatments (mean = 29.4%) and the final density was 29.3 ind./L. Previous studies on black sea bass testing varying dietary DHA/ARA ratios produced much higher survival (62%) by d24ph in treatments high in DHA (10% TFA) and ARA (6%) (Rezek et al., 2005). However, these authors used a lower initial stocking density of 40 ind./L (Rezek et al., 2005), and final density was 24.8 ind./L, similar to the current study and suggesting that stocking density plays an important role in overall survival to metamorphosis.

In this study, no significant differences in survival were observed for larvae fed live prey enriched to contain different ARA levels within a relatively broad range of 0 – 12% (TFA), or with the commercial booster Algamac 2000. However, there was a great deal of replicate variability in all treatments, which may have masked any treatment effects on survival. The effects of dietary ARA on survival in striped trumpeter (Bransden et al., 2004) and turbot (Estevez et al., 1999) were similar to those observed in

black sea bass. Differences in survival of larval striped trumpeter fed increasing levels of dietary ARA (1.33 – 11.22 mg/g ARA as DW) were also obscured by high variability (as high as 40% variation) among replicate units (Bransden et al., 2004). In larval turbot, varying dietary ARA (3 – 15% ARA as TFA) also produced no treatment effects on survival up to day 43 post – hatching (Estevez et al. 1999).

In contrast to black sea bass, striped trumpeter and turbot, marked improvements in survival in larval summer flounder (Willey et al., 2003) and gilthead sea bream (Koven et al., 2003) fed rotifers and Atemia enriched to contain high dietary ARA were seen as early as 2 weeks after pre – metamorphosis. It is possible, therefore, that the effects of dietary ARA on larval survival in black sea bass are not apparent until well after the pre – metamorphic stages, when the effects of ARA on the metamorphic process combined with increased handling stresses (such as harvesting and transferring fish) are evident (Koven et al., 2003). Koven et al. (2001) suggested that the earlier the larvae are fed ARA the better prepared the larvae are during later life stages. Summer flounder, fed rotifers enriched with various levels of ARA (0 to 12%) showed no differences in survival by day 18 post – hatching (Willey et al., 2003). These authors divided the larvae from 0 and 6% ARA enriched rotifer treatments into sub groups, involving an acute stress induced by tank transfer, and then fed ARA enriched Artemia ranging from 0 to 12% through d47ph. Under this acute stress, larvae fed 6% ARA enriched rotifers showed better survival by d47ph than larvae fed 0% ARA enriched rotifers; however no differences were observed between 0 – 12% ARA enriched Artemia treatments in either the 0 or 6% ARA rotifer enrichments (Willey et al., 2003). These results also suggested

that ARA supplementation during the early larval stages is crucial to survival in later post-metamorphic stages.

Gilthead sea bream fed a diet high in ARA (52% TFA) showed better survival (42.9%) on day 35 post – hatching after an acute stress (tank transfer on d19ph) than larvae fed either a commercial diet ALG (31.0%) or a high-DHA diet deficient in ARA (17.5%) (Koven et al., 2001). Larval gilthead sea bream (d35ph) with very low levels of tissue ARA (2.9 mg/g DW) (originating from DPAn-6 in ALG) had significantly lower mortalities than those fed a diet deficient in ARA (1.0 mg/g DW) and high in DHA, demonstrating that low levels of ARA increased survival (Koven et al., 2001). In a more recent study, Koven et al. (2003) demonstrated the effects of ARA on larval stress in d32 and d42ph gilthead sea bream, where lower mortalities were observed in larvae fed high levels of ARA (25% ARA as TFA) within a range of 0 to 25% TFA under the acute stress of tank transfer. Larvae fed the same treatments and subjected to the chronic stress of continuous salinity changes, however, had fewer mortalities on d32ph in low ARA treatments (0 and 12.5% TFA), but no differences among treatments were observed on d42ph in any of the treatments (0-25% ARA). The results suggested that high dietary ARA does not promote survival under chronic salinity stress (elevated stress over extended periods of time) but increases the larva's ability to cope with an acute handling stress. Careful attention to the quality of water should reduce the likelihood of a chronic stress situation in a hatchery. In addition, dietary ARA supplementation could increase larval resistance to acute handling stressors commonly encountered during hatchery production.

Hypersaline Stress Resistance

In this study, there were no significant differences among dietary treatments on the ability of black sea bass larvae to tolerate hypersaline (55 ppt) stress. These results are similar to a previous study, where black sea bass fed ARA at levels from 0 to 6% TFA and DHA levels of 0 and 10% TFA showed no significant differences in stress resistance when challenged with an acute salinity increase to 65 ppt (Rezek et al., 2005). These authors suggested that exposure to this high salinity caused rapid mortality in all treatments and therefore did not allow treatment effects on hypoosmoregulatory ability to be resolved (Rezek et al., 2005). Similar to the black sea bass, larval striped trumpeter (d23ph) showed no relationship between dietary ARA levels from 0 – 36% and hypersaline (55 ppt) stress tolerance (Bransden et al., 2004).

Many studies have used hypersaline stress evaluation as a tool to evaluate larval quality in marine finfish, including the Asian sea bass (Dhert et al., 1990), Japanese flounder (Furuita et al., 1999), striped bass (Harel et al., 2001) and summer flounder (Willey et al., 2003). For the most part, these studies suggest ARA plays an important role in improving stress resistance if fed at proper levels. For example, striped bass raised in low salinity water (2 – 6 ppt) and fed the lowest levels of ARA (4.7 mg/g DW) produced the lowest survival during hypersaline (25 ppt) stress evaluation, while intermediate levels of ARA (15.4 mg/g DW) increased stress tolerance, but further increases in dietary ARA (19.6 mg/g DW) reduced survival (Harel et al., 2001). Summer flounder fed increasing dietary ARA ranging from 0 to 12% showed improved

hypersalinity tolerance on both d18ph (2 h in 70 ppt) and d47ph (2 h in 80 ppt) in larvae fed an intermediate level of ARA (6% TFA) (Willey et al., 2003).

It appears that the method used to evaluate stress resistance and larval quality in black sea bass in this study was ineffective. As previously suggested (Rezek et al., 2005) a salinity of 55 ppt was lethal for black sea bass larvae (d24ph), overwhelming the larva's ability to adapt to the acute salinity stress and causing rapid death within a period of one hour. Van Anholt et al. (2004) found larval gilthead sea bream fed two levels of dietary ARA (1.5, 7.5 mg/g DW) showed a similar response to a decrease in salinity (from 42 to 25 ppt) with elevated $\text{Na}^+ \text{K}^+$ ATPase (NKA) activity after 120 min. Although the NKA activity increased initially, it did not stay elevated in the low ARA (1.5 mg/g DW) fed larvae after 24 h, whereas NKA remained elevated and whole body sodium content decreased in the high ARA (7.5 mg/g DW) fed larvae, suggesting that increased dietary ARA was associated with increased hypoosmoregulatory ability.

European sea bass (Jensen et al., 1998) and killifish (Scott et al., 2004) responded to salinity transfer by increasing the mRNA expression of NKA after 24 h followed by an increase in NKA activity after 48 h. The results of the present study suggested that NKA expression was significantly elevated after 24 h of exposure to 43 ppt in larvae fed 6 – 12% ARA as TFA, but not in 0% ARA- or ALG-fed larvae. Based on these data, we recommend that future studies on stress resistance in black sea bass use a sub-lethal salinity challenge of 45 – 50 ppt. This lower salinity will allow the larva's hypoosmoregulatory system, including the development of ionic transport enzymes (e.g. $\text{Na}^+ \text{K}^+$ ATPase) to adapt to the salinity stress and therefore display differences in osmoregulatory function among larvae fed different dietary treatments. Furthermore,

NKA activity or mRNA expression levels should be monitored closely over extended periods (> 24 h) of time following salinity challenge to determine possible correlations between dietary ARA, stress resistance, hypo-osmoregulatory ability and survival as previously demonstrated in gilthead sea bream (Koven et al., 2003; Van Anholt et al., 2004).

mRNA expression of Na⁺ K⁺ ATPase

This is the first study to demonstrate the role of arachidonic acid in mRNA expression of NKA. Our results indicate that increased dietary ARA and ARA in the tissues of black sea bass larvae (> 6.7% TFA) significantly increased the relative expression of NKA 24 h after exposure to a sub-lethal increase in salinity of 8 ppt (from 35 to 43 ppt). Van Anholt et al. (2004) found larval gilthead sea bream fed dietary ARA (7.5 mg/g DW) were better able to tolerate a sub-lethal decrease in salinity (from 42 to 25 ppt) than larvae fed lower levels of ARA (1.5 mg/g DW). In both ARA treatments, NKA activity increased initially but did not stay elevated after 24 h in the low ARA treatment (1.5 mg/g DW), while NKA remained elevated and whole body sodium content decreased and was much lower in the high ARA treatment (7.5 mg/g DW), suggesting that hypo-osmoregulation was impaired in the low ARA treatment (Van Anholt et al., 2004). Larvae in both high and low ARA treatments remained hypoosmotic after salinity transfer. However, while no differences in whole body sodium were noted in the low ARA (1.5 mg/g DW) fed larvae over a 24 h period post-transfer, whole body sodium in the high ARA fed larvae decreased and was significantly lower than in the low ARA-fed

larvae, suggesting increased NKA activity and extrusion of sodium. Grouper (*Epinephelus coioides*) larvae (d20ph) subjected to a salinity increase of 8 ppt (from 32 to 40 ppt) showed low NKA activity and reduced osmoregulatory ability causing increased mortality at 25° C and complete mortality at 30° C (Caberoy and Qunitio, 2000). These authors suggested that this decrease in NKA activity after 12 h was in part due to an increase in NKA activity to its maximum potential which was unsustainable and ultimately resulted in overload and failure of the NKA enzyme (Caberoy and Qunitio, 2000).

Tissues of cultured European sea bass and gilthead sea bream were found to have low ARA compared to wild-caught fish, suggesting that the dietary ARA levels during culture of these fish were inadequate (Koven et al., 2001). Optimizing dietary ARA may increase the potential for successful rearing of a candidate species, especially when exposed to acute stressors, such as an unexpected change in rearing salinity. It is well known that ARA is a preferred substrate for the synthesis of eicosanoids such as prostaglandins (PG's) with an enzyme called cyclooxygenase (Tocher et al., 1996; Sargent et al., 1999; Bell et al., 2003b).

ARA is typically found in phospholipids which make up cellular membranes and can be converted to PG's in response to changes in extracellular ion concentrations, potentially increasing the sensitivity of the hypothalamus-pituitary-interrenal axis (HPI axis) in fish (Bell et al., 1983; Linares and Henderson, 1991; Van Anholt et al., 2004). In mammals, ARA modifies the release of cortisol from the adrenals of the hypothalamus-pituitary-adrenal (HPA) axis, but its precise function in fish is not clear (Lands, 1991, Hockings et al., 1993, Nye et al., 1997; VanAnholt et al., 2004). Tocher et al. (2000)

found a relationship between PG's and osmoregulation in Atlantic salmon smolts, where PG's appear to modulate the sensitivity of the HPI axis to the production of cortisol similar to how PGE₂ regulates cortisol's release in the HPA-axis in mammals.

PG's play a key role in electrolyte balance and cellular ion fluxes which are essential in all animal cells (Mustafa and Srivastava, 1989). Marine finfish continuously drink water because they are hypo-osmotic to sea water and must therefore replace osmotic water losses to their environment, and actively extrude ions from tissues. The regulation of ions in response to osmotic gradients helps to keep inter-cellular fluids at homeostasis with extra-cellular fluids (Mustafa and Srivastava, 1989; Scott et al., 2004). Bell et al. (1994) found increased PGE₂ production in turbot astroglial cell cultures supplemented with 25 μM ARA (4 days) after calcium ionophore stimulation.

In fish, increased cortisol stimulates increased NKA activity and chloride cell density which are essential components in the active transport of ions across cell membranes (Dang et al., 2000). Since cortisol is regulated by PG's synthesized from ARA, it is likely that ARA has an affect on NKA and ultimately the osmotic stress response in fish. Koven et al. (2003) found that elevated levels of cortisol caused by extended periods of induced stress (daily salinity fluctuations) in high ARA (25% TFA) fed larvae, reduced the larva's immune response. So, too much dietary ARA will stimulate too much cortisol production and negatively impact larval performance. Striped bass fed dietary ARA and subjected to a hypersaline challenge, showed elevated tissue ARA, cortisol and increased hypersaline stress tolerance (Harel et al., 2001). Increases in cortisol have been found to increase the specific activity of NKA and the differentiation of Cl⁻ cells which contain NKA and are primarily responsible for ion regulation in fish (Dang et al., 2000).

NKA is located in the membranes of most cells and is responsible for the active transport of sodium out of the cell and potassium into the cell (Scott et al., 2004). Gilthead sea bream fed high dietary ARA showed increased NKA activity accompanied by increased osmoregulatory ability after being subjected to an acute hyposaline-transfer stress (from 42 to 25 ppt) (Van Anholt et al., 2004). Similar to the black sea bass, both killifish (Fundulus heteroclitus) (Scott et al., 2004) and European sea bass (Dicentrarchus labrax) (Jensen et al., 1998), showed increases in mRNA expression of NKA 24 h after transfer to a higher strength sea water. There is a lag time of 2-3 days in most teleosts between mRNA expression of NKA and increased NKA specific activity. European sea bass and killifish showed an increase in NKA activity 48 h following salinity transfer (Jensen et al., 1998; Scott et al., 2004). Increasing the salinity (from 0 to 31 ppt) during the smoltification process in Atlantic salmon (Salmo salar) caused increasing NKA expression levels after 24 h (D'cotta et al., 2000). In contrast, Singer et al. (2003) found that direct cortisol injection in Atlantic salmon smolts of different strains subjected to salinity transfer did not show differences in mRNA NKA expression after 6 days. Based on D'cotta et al. (2000) it is likely that the analysis was made too long after injection and that the up-regulation of mRNA signal transduction was missed.

While the optimal level of dietary ARA in black sea bass is still not clear, its effects on mRNA expression were clearly demonstrated in this study. Based on the optimization of mRNA expression of NKA, we recommend that ARA should be used at an intermediate level (6-12 % TFA) in combination with adequate levels of other EFA's, DHA and EPA (at least 2:1). This combination would promote optimal growth, survival and hypersaline stress resistance in black sea bass larvae.

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