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Effects of dietary arachidonic acid on larval performance, fatty acid profiles, stress resistance, and expression of Na^+/K^+ ATPase mRNA in black sea bass *Centropristis striata*

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ABSTRACT

Dietary arachidonic acid (ARA, 20:4n-6) supplementation on larval performance, tissue fatty acid profiles, resistance to hypersaline stress and expression of Na^+/K^+ ATPase mRNA was studied in black sea bass *Centropristis striata* from the first feeding through metamorphosis at 24 days post-hatching (d24ph). Thirty 15-L aquaria were stocked with d1ph yolksac stage larvae at 100 ind/L. Salinity (34 g/L), temperature (22 °C), photoperiod (18L:6D), light intensity (1000 lx), diffused aeration (100 mL/min) and D.O. (>5 mg/L) were held constant. Non-viable microalgae *Nannochloropsis oculata* was maintained at 300,000 cells/mL as background. To evaluate the effects of dietary arachidonic acid (ARA) supplementation, live prey, rotifers *Brachionus rotundiformis* and *Artemia* sp., was 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 a premium commercial fatty acid booster (26% DHA, 0% ARA). Rotifers were fed from d2 to d20ph at 10–23 ind/mL, while *Artemia* were fed from d18 to d22ph at 0.5–3 ind/mL. On d24ph, larval fatty acid profiles reflected dietary levels, and no significant ($P>0.05$) differences in larval growth (notochord length, wet and dry wt.), survival (range = 24.3–32.7%), or hypersaline stress resistance (ST-50 = 27.1–31.8 min) among treatments were evident. However, larvae fed diets supplemented with ARA (6–12% TFA) demonstrated a significant ($P<0.05$) increase in Na^+/K^+ ATPase mRNA 24 h after a sublethal salinity (43 g/L) challenge, whereas larvae fed 0% ARA and the commercial diet (devoid of ARA) showed no significant increase. The results suggested 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.

1. Introduction

Black sea bass *Centropristis striata* (family Serranidae) is a high-value marine finfish that inhabits continental shelf waters from Maine to northern FL that supports important commercial and recreational fisheries (Mercer, 1989). This species is overfished in the area south of Cape Hatteras (NCDMF, 2009). A high demand placed on the commercial fishery to supply both local and niche markets has stimulated an interest in developing techniques for aquaculture of black sea bass. Protocols for controlled breeding of black sea bass are well developed (Berlinsky et al., 2004; Denson et al., 2007; Howell et al., 2003; Watanabe et al., 2003, in press). A constraint to the artificial propagation of this species is reliable larviculture techniques to provide healthy fingerlings to grow-out operations. A number of studies have investigated the optimum environmental parameters for production of black sea bass fingerlings, including temperature, photoperiod, salinity and light intensity (Berlinsky et al., 2004; Copeland and Watanabe, 2006). Little or no experimental data is available on larval nutrition.

Available live feed organisms for marine fish larvae, rotifers and *Artemia*, are generally deficient in essential fatty acids and must be enriched with these components before feeding to the larvae. Essential fatty acids (EFAs) are a class of lipids that the fish is unable to synthesize *de novo* (Kanazawa, 2003; Tacon, 1990) and are critical to growth and survival. Known EFAs 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 (Furuita et al., 1998; Kanazawa, 2003; Mourente and Tocher, 1992; Valentine and Valentine, 2004; Voet et al., 1999). If not provided sufficiently in the

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diet, DHA is synthesized from EPA which is synthesized from linolenic acid (LN, 18:3n-3) through a process of desaturation and carbon chain elongation (Tacon, 1990; Voet et al., 1999), while ARA is synthesized from linoleic acid (LL, 18:2n-6). ARA and EPA are precursors to eicosanoids such as prostaglandins, leukotrienes and thromboxanes (Bell and Sargent, 2003; Van Anholt et al., 2004; Voet et al., 1999) that are released during stress and the inflammatory response (Van Anholt et al., 2004). In contrast to freshwater fish, the functional activities of the enzymes Δ5 desaturase and C18–20 elongases necessary for the synthesis of DHA, EPA or ARA (Kanazawa et al., 1979; Tocher and Ghioni, 1999) from their precursor fatty acids LL and LN (Kanazawa, 2003; Lavens et al., 1995; Tacon, 1990) are low in marine fish.

Previous studies have demonstrated the importance of high levels of DHA compared to EPA in the diets of larval marine fish (Rainuzzo et al., 1997; Tocher and Sargent, 1984; Watanabe, 1993; Watanabe et al., 1989). More recent studies have also demonstrated the importance of ARA in larval growth, survival, and stress resistance in different species of marine finfish. Striped bass *Morone saxatilis* fed dietary ARA and subjected to a hypersaline challenge showed elevated tissue ARA and increased hypersaline stress tolerance (Harel et al., 2001). In summer flounder *Paralichthys dentatus* (Willey et al., 2003) and gilthead seabream *Sparus auratus* (Koven et al. 2001), dietary ARA supplementation improved growth, survival and stress resistance. In *S. auratus*, increasing dietary ARA within the range of 0 to 25% TFA increased cortisol levels of larvae exposed to chronic hyperosmotic and hyposmotic stress (Koven et al., 2003). Larvae fed an intermediate level of ARA (12.5%) showed higher survival when subjected to salinity changes than larvae fed the highest or lowest level of ARA. These workers suggested that 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). In black sea bass larvae fed diets containing 10% DHA, increasing ARA within the range of 0–6% improved growth and survival from first feeding through metamorphic stages (Rezek et al., 2010).

Osmoregulation in larval fish is accomplished by Na^+/K^+ ion-transport pumps within cells in the integument until the gills and intestinal osmoregulatory systems develop (Rombough, 2007). The enzyme Na^+/K^+ ATPase (NKA) mediates the active secretory functions of chloride cells in juvenile fish by generating a chemical gradient for ion transport. Osmoregulatory ability can be evaluated by measuring the expression of NKA messenger RNA (mRNA), with a higher mRNA level indicating the elaboration of the ion transfer pump and better osmoregulatory ability (Carrier, 2006; Ostrowski et al., 2011; Scott et al., 2004; Scott and Schulte, 2005). In the silver sea bream *Sparus sarba* increases in NKA mRNA levels during the first 35 days of larval development were reported (Deane et al., 2003), but the role of larval nutrition on elaboration of NKA mRNA has not been investigated. The objectives of this study were to evaluate the effects of dietary ARA supplementation (0–12%) in black sea bass larvae on growth, survival, tissue fatty acid profiles, resistance to hypersaline challenge, and the expression of NKA mRNA as an index of hypo-osmoregulatory ability.

2. Methods

2.1. 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 tanks (vol. = 2134 L) supported by biofilters, ultraviolet sterilizers, heat pumps and fluorescent lights to control water quality, temperature and photoperiod. Seawater (34 g/L) was pumped from the adjacent Atlantic intracoastal waterway and was filtered (1-μm) and UV-sterilized. Volitional spawning was induced in a mature post-vitellogenic stage females using pelleted luteinizing hormone releasing hormone-analog (Berlinsky et al., 2000; Berlinsky et al., 2004; Watanabe et al., 2003, Watanabe et al. in press). Eggs were collected from broodtanks and viable (buoyant) eggs were separated from nonviable (non-buoyant) eggs. Fertilized eggs were incubated in a 125-L tank at 34 g/L and at 18 °C until hatching.

2.2. Experimental system

The experimental system consisted of four temperature controlled water baths (213-L) 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, with eight tanks in each water bath. A light hood over each bath contained two 40-watt full-spectrum fluorescent light bulbs controlled by timers for photoperiod manipulation and was adjusted vertically for surface light intensity.

2.3. Experimental design

To begin the experiment, newly hatched yolk-sac larvae (approximately 52 h post-fertilization) were distributed from the incubator to each experimental tank at a density of 100 ind/L. 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 enriched with a popular commercial fatty acid booster for marine finfish larvae (Algamac 2000, Aquafauna Bio-Marine, Hawthorne, CA, USA), containing 26% DHA, 10.6% docosapentaenoic acid (DPAn-6, 22:5n6), 0.6% EPA and 0% ARA, to establish performance of larvae using current industry standard procedures and to evaluate retro conversion of DPAn-6 to ARA in live prey and in black sea bass larvae. Larvae were reared through metamorphosis to 24 days post-hatching (d24ph).

2.4. Experimental conditions

Environmental conditions, including salinity (35 g/L), temperature (22 °C) (Berlinsky et al., 2000), pH (7.3–8.0), photoperiod (18L:6D), light intensity (1000 lx) (Copeland and Watanabe, 2006), diffused aeration (100 mL/min) and dissolved oxygen (>5 mg/L) were monitored daily in each tank and maintained at prescribed levels throughout the experiment. Background micro-algae *Nannochloropsis oculata* non-viable paste (Reed Mariculture, Campbell, CA, USA) was added daily to maintain a density of 300,000 cells/mL (Berlinsky et al. 2004). To maintain water quality, 5 L (33.3% of the tank volume) was siphoned daily and replaced with filtered and sterilized seawater. Surface oils were removed daily using a paper towel.

2.5. Feeds

S-type rotifers *Brachionus rotundiformis* were grown at the UNCW Aquaculture Facility on non-viable microalgae *N. oculata* paste (Reed Mariculture, Campbell, CA, USA) (Bentley et al., 2008). Rotifers were enriched with the treatment emulsions for 8 h before feeding to the fish larvae. *Artemia* metanauplii hatched from decapsulated cysts were enriched with the treatment emulsions for 16 h before feeding to the larvae. Prey fatty acid profiles were identified by gas chromatography (Tables 1 and 2).

Rotifers were introduced to the rearing tanks on d2ph at a density of 10/mL until first feeding on d3ph, increasing to 15/mL by d3ph and then to a maximum of 23/mL by d18ph. *Artemia* were co-fed with rotifers on d18ph at a density of 0.5/mL. On d20ph, *Artemia* density was increased to 2/mL, and rotifer feeding ceased. On d22ph, *Artemia* density was increased to 3.0/mL for the remainder of the experiment.