Live prey enrichment and artificial microdiets for larviculture of Atlantic red porgy Pagrus pagrus

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A B S T R A C T

In the first experiment the effects of rotifer enrichment and feeding frequency on larval performance of red porgy Pagrus pagrus were studied. Larvae (2 days post-hatching ~2 dph) were fed s-type rotifers (~20 rotifers/mL) enriched with one of the four different treatment media: Rotifer Diet (microalgae Nanochloropsis oculata and Tetraselmis chuii), DHA Protein Selco, Algamac 3000 (Schizochytrium sp.) and Algamac + ARA (arachidonic acid). Larvae were fed daily at full ration or twice daily at half ration. Larval growth and survival (mean = 22.8%) were satisfactory through 16 dph under all treatments; however, resistance to hyposaline challenge (Survival Activity Index = SAI) was positively correlated (P < 0.01) with DHA concentration of rotifers, and SAI appeared highest in the Algamac + ARA treatment. In the second experiment the effects of Artemia enrichment on larval performance were compared from 18 dph through pre-metamorphosis (33 dph). Larvae were fed Artemia (0.5–3.0/mL) enriched with two different media Algamac 3000 and DC DHA Selco, or unenriched Artemia (control). Both media improved DHA levels in Artemia and growth and survival (36.7–54.6%) of larvae, while larvae fed unenriched Artemia showed poor growth and survival (5.2%). In the third experiment a University of North Carolina Wilmington microbound diet (MBD) and two commercial microdiets (Gemma Micro and Otohime) were evaluated. The MBD contained different protein sources (i.e., menhaden, squid and krill meal, soy protein concentrate) and attractants. Beginning 16 dph, live feeds and microdiets were co-fed to three treatment groups of larvae: (1) Gemma, (2) MBD, and (3) Otohime. Larval performance on the UNCW-MBD was comparable to the commercial microdiets, with no significant differences in larval survival, DHA, or total n–3 PUFA content through 32 dph. Results delineate more effective rearing protocols for larviculture of Atlantic red porgy juveniles.

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1. Introduction

The red porgy Pagrus pagrus, also known as sea bream, silver snapper, or pink snapper, is a valuable marine finfish in the family Sparidae, inhabiting the Mediterranean Sea, the eastern Atlantic (from the British Isles to Senegal), and the western Atlantic (from North Carolina to Mexico and from Venezuela to Argentina) (Morris et al., 2008). An important component of the snapper–grouper complex in the coastal Atlantic off the SE US (particularly NC and SC), red porgy populations have declined severely (SAFMC, 2006, 2010; Vaughan and Prager, 2002), and stringent requirements for capture of red porgy have been established (SAFMC, 2006, 2010). Due to declining natural populations, high market value, and suitability for intensive culture in tanks and cages, red porgy are considered a promising species for farming in the Mediterranean (Kolios et al.,...
Studies to date indicate that, aside from high early mortality related to swim bladder inflation (Mihelakakis, 2001b; Papandroulakis et al., 2004), red porgy are a durable species under hatchery conditions (Roo et al., 1999; Mihelakakis et al., 2001b; Papandroulakis et al., 2004; Buke et al., 2005; Morris et al., 2008; Roo et al., 2010). Significant progress has been made in understanding the spawning and larval culture requirements of Atlantic red porgy (Morris et al., 2008; Ostrowski et al., 2011) and producing post-metamorphic juveniles in the laboratory (Morris et al., 2008). However, greater knowledge of practical larval rearing technology is needed for reliable mass propagation and to remove an important constraint to commercial farming of this species (Cavalin and Weirich, 2009; Andrade et al., 2012).

Live feeds commonly used in marine finfish hatcheries, particularly rotifers Brachionus spp. and brine shrimp Artemia spp., are inadequate and variable in nutritional value. Therefore, it is critical to enhance these prey items with essential nutrients (e.g., fatty acids) before feeding them to the fish larvae (Izquierdo et al., 1989; Hernandez-Cruz et al., 1999; Sargent et al., 1999; Sorgeeos et al., 2001). Previous studies have shown growth performance of larval red porgy is affected by levels of highly unsaturated fatty acids (HUFAs) docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3) provided in live prey (Hernandez-Cruz et al., 1999; Roo et al., 2009). More recent studies have shown that levels of the n-6 HUFA arachidonic acid (ARA, 20:4n-6) in live prey can also affect growth and stress resistance in marine finfish larvae (Koven et al., 2001b; Rezek et al., 2010; Carrier et al., 2011; Watanabe et al., 2014). Numerous commercial media have been formulated to boost HUFA profiles and other essential nutrients in live prey. These include preserved microalgae concentrates, yeast and lipid powders and semi-moist pastes, and freeze-dried heterotrophically-grown microalgae. These products yield different nutritional biochemical profiles in live prey which markedly affect growth and stress resistance of the marine fish larvae fed enriched prey (Faulk and Holt, 2005; Faulk et al., 2005; Garcia et al., 2008; Cavalin and Weirich, 2009).

Experimental data comparing the efficacies of popular live prey enrichment media for larval culture of most marine finfish is fragmentary and is of considerable practical importance. No studies have compared commercial enrichment media on larval growth performance and stress resistance in red porgy.

Available information suggests that larval growth and production costs can be affected by feeding frequency of live prey, which affects prey quality, digestion and assimilation by larvae, as well as feed waste and water quality. The optimum feeding frequency and rate vary depending on species, fish size and rearing system (Rabe and Brown, 2000; Cho et al., 2003; Russo, 2013; Woolley and Partridge, 2015). Due to high labor requirements and costs for raising live prey, minimizing or eliminating the use of live feeds by substituting artificial formulated microdiets (MDs) will also be important for more cost-effective production protocols in marine finfish hatcheries (Yufera et al., 1999; Baskerville-Bridges and Kling, 2000; Koven et al., 2001a; Cahu and Zambonino-Infante, 2001; Teshima et al., 2004; Kolkovsky, 2009; Salhi and Bessonart, 2012; Andrade et al., 2012; Alam et al., 2013). Zein-bound microdiets were successfully in the larval rearing of red sea bream Pagrus major a congener of the red porgy (Kanazawa et al., 1989; Teshima et al., 2000; 2004). Zein is a high molecular weight, non-toxic, edible protein with amphoteric and thermoplastic properties. Zein is soluble in 40–90% alcohol, making preparation of zein microparticles less toxic compared with preparation methods that involve the use of toxic solvents. Ethanol can also be easily removed by evaporation or spray-drying to form zein-bound complex particles. Furthermore, amino acids and many other low molecular water-soluble materials are significantly less soluble in ethanol than pure water, reducing the leakage loss of core materials during particle preparation (Langdon et al., 2007). Experimental studies on the use of artificial microdiets for weaning larvae from live prey, including the efficacies of zein-bound and commercially available products, are needed to advance reliable large-scale hatchery production of red porgy fingerlings.

The overall objective of this study was to contribute to optimizing feeding protocols during hatchery rearing of red porgy. Specific objectives were to (1) optimize enrichment methods for live feeds by comparing practical enrichment media and feeding frequency of live prey on larval performance, and (2) to minimize reliance on live prey through artificial microdiets (MDs) for co-feeding and early weaning.

2. Methods

2.1. Experiment 1: comparison of rotifer enrichment media

2.1.1. Experimental animals

Red porgy broodstock (N = 20, mean weight = 373 ± 2 g, mean total length = 306 ± 4.7 mm) were collected by hook and line off the coast of North Carolina and maintained in a recirculating 31-m³ round tank at the Center for Coastal Fisheries and Habitat Research, National Oceanic and Atmospheric Administration (Beaufort, NC, USA). Broodstock were held under conditions simulating seasonal offshore bottom temperatures off North Carolina from 27–33° C (Parker and Dixon, 1998) and under ambient photoperiod. Broodstock spawned volitionally in captivity (Morris et al., 2008) during their natural spawning season (January–April), and eggs were collected by filtering surface water from the spawning tank through a 505-μm mesh nitex net. Eggs were transferred to the University of North Carolina Wilmington Center for Marine Science (UNCW-CMS) Aquaculture Facility (Wrightsville Beach, NC, USA) in plastic bags containing oxygenated seawater. Eggs were placed in a conical plexiglass tank (15-L volume) in 32 g/L seawater where viable (buoyant) eggs were separated from nonviable (non-buoyant) eggs before the experiment. Seawater for larval culture experiments was pumped from the Atlantic Intracoastal Waterway adjacent to Wrightsville Beach, passed through a 1-μm filter and UV-treated before being added to rearing tanks.

2.1.2. Experimental system

Red porgy larvae were reared in 15-L cylindrical, black polyethylene tanks held in four temperature-controlled water baths (213–L) in a controlled-environmental laboratory. Light intensity at the surface of each tank was 500 lx (492.3 ± 3.9) (Ostrowski and Watanabe, UNCW, unpublished data) supplied by two 40-W full-spectrum fluorescent light bulbs suspended over each table. Photoperiod was 16L:8 D starting at 0700 h and ending at 2300 h. Extraneous light was excluded by black curtains surrounding the water baths.

To begin the experiment, yolksac larvae (0 days post-hatching = 0 dp) were initially stocked into each tank at a density of 60 larvae/L at 34 salinity and 19 °C. Temperature was adjusted 1 °C per day after hatching until an experimental temperature of 21 °C was reached by 2 dp (Ostrowski et al., 2011). Air was supplied to each tank through an air diffuser positioned at the bottom center of each tank. Airflow was 90 mL/min to avoid injury from excessive turbulence (Mangino and Watanabe, 2006).

2.1.3. Experimental design

Using a standardized larval feeding regime developed for red porgy at UNCW (Morris et al., 2008; Ostrowski et al., 2011), different rotifer enrichment media were tested concurrently. First-feeding...
stage larvae were fed, beginning 2 dph and continuing through 18 dph, s-type rotifers *Brachionus rotundiformis* (5–20 rotifers/mL) enriched with one of following four different treatment media: Rotifer Diet a combination of the microalgae species *Nannochloropsis oculata* and *Tetraselmis chuii* (0.22% total fatty acids DHA, 40.72% EPA, 4.34% ARA; Reed Mariculture, Campbell, CA), DHA Protein Selco (25 mg/day DHA, 10 mg/day EPA, 0 ARA; INVE Aquaculture, Belgium), Algalac 3000 (heterotrophically-grown *Schizochytrium sp.*; 41.3% DHA, 14.72% EPA, 1.3% EPA, 0% ARA; BioMarine Aquafana, CA, USA) and Algalac+ARA (mix of 75% Algalac 3000 and 25% Algalac-araachidonic acid; 2.4% DHA, 0% EPA, 0% ARA, 37% ARA, BioMarine Aquafana, CA, USA). Rotifers were fed at a concentration of 5 per mL on 2 dph, increasing to 7, 10, 12, 15, and 20 per mL by 4, 7, 9, 12 and 14 dph, respectively.

To study the combined effects rotifer enrichment and feeding frequency on larval performance, larvae under the four different enrichment treatments were either fed once daily (0830 h) at full ration (20 rotifers/mL), or twice daily (0830 and 1600 h) at half ration (10 rotifers/mL). Four replicate units were assigned to each treatment combination of enrichment media and feeding frequency in a 4 × 2 factorial design. Rotifers were cultured on RotiGrow (DHA 0%, EPA 22.5%, ARA 3.2%, HUFA 36.0% TFA, Reed Mariculture Inc., Campbell, CA) in a continuous culture system (Bentley et al., 2008) and were harvested daily from the system and enriched for 12 h according to protocols recommended by the manufacturer before being fed to the larvae. After the first (0830 h) feeding, enriched rotifers were held at 10 °C to maintain nutritional profile before the second (1600 h) feeding. The fatty acid profiles of rotifers enriched with these different media are summarized in the Results section.

### 2.1.4. Experimental conditions

Water was exchanged daily (33%) by siphoning 5 L from each tank at 0600 h and replacing with filtered seawater at 22 °C. Water exchange was completed between 0600 and 0830 h before the first feeding. From d2 to d16 ph, a non-viable, condensed microalgae *N. oculata* paste (Reed Mariculture, Campbell, California, USA) was added to each tank at a concentration of 750,000 cells/mL as background. The water surface of each tank was “skimmed” daily using a paper towel to remove oil and debris.

### 2.1.5. Water quality

Temperature (22 ± 0.1 °C), salinity (34 ± 1), D.O. (7.21 ± 0.08 mg/L), pH (range = 8.10–8.40) (YSI 560, Yellow Springs, Ohio, USA), and light intensity (492 ± 4 lx) were measured twice daily (0900 and 1600 h) from alternating replicate tanks per treatment. Ammonia (0.2 ± 0.08 mg/L) was tested twice a week (HACH DR 850, Loveland, Colorado, USA; 0.02 ± 0.005 mg/L) and airflow to each tank was tested daily using a flow meter (Gilmont Instruments Inc., Barrington, Illinois, USA; ±1 mL/min) and adjusted as necessary.

### 2.1.6. Growth and survival

Larval growth and survival were monitored using volumetric methods (Henne and Watanabe, 2003; Moustakas et al., 2004; Mangino and Watanabe, 2006). Larvae were sampled by plunging a 2-cm PVC pipe, 46 cm in length, into the tank and then transferring the water and larvae collected to a graduated cylinder. Prior to sampling, airflow was increased to uniformly distribute the larvae in tanks. Larvae were randomly sampled from each replicate tank on 1, 7, 10, and 16 dph at approximately 0730 h. The number of fish were counted and divided by the water volume removed during sampling to determine survival. After anesthetizing the larvae with 0.5 mg/L of 2-phenoxyethanol, larvae were placed in a Petri dish and notochord lengths (NL) were measured using an ocular micrometer on a dissecting microscope (Meiji Techno Co., Ltd., Japan). Dry weight was determined using an electrobalance (±0.01 mg) (Sartorius, Goettingen, Germany) by placing 10 larvae on a pre-weighed microscope slide after they were poured onto a 23-µm mesh screen, which was blotted with a sponge from underneath to remove excess water. The slides were then placed into a laboratory oven at 60 °C for 72 h and then reweighed to determine dry weights of larvae.

### 2.1.7. Hyposaline stress resistance

At the end of the experiment (16 dph), a hyposaline stress test was performed on the remaining larvae from each treatment to measure their ability to withstand an abrupt decrease in salinity to 24 % with conditions of zero feeding. Using volumetric methods, survival was monitored every 12 h for 48 h. A survival activity index (SAI) to test larval quality was calculated using the following equation:

\[
SAI = \frac{1}{N} \sum_{i=1}^{k} \left( N - h_i \right) \times i
\]

where \( N \) is the total number of initial larvae, \( h_i \) is the cumulative mortality by the \( i \)th hour, and \( k \) is the number of hours that have elapsed until all larvae have died or the experiment ended (Matsuo et al., 2006).

### 2.1.8. Fatty acid composition

Fatty acid composition of the enriched rotifers from each treatment at the beginning and at the end of the experiment was determined by first extracting total lipids into chloroform:methanol (Folch et al., 1957). One milliliter of a 0.001 g/mL-1 solution of C19:0 fatty acid was added to each sample as an internal standard. Lipid fatty acids were converted to their methyl esters (FAMEs) for GC analysis by refluxing the concentrated lipid sample in 1.0 mL of 0.5 M NaOH/MeOH for 30 min, followed by addition of 1.5 mL of boron trifluoride-methanol (BF3) and refluxing for an additional 30 min. The FAMEs were extracted into hexane, concentrated and dissolved in 1 mL of chloroform. GC analysis was performed on a HP-6890 Gas Chromatograph using a 25 m × 0.25 mm HP-5 capillary column with FID detection (Department of Chemistry and Biochemistry, UNCW). Helium was used as the carrier gas. The column temperature profile was: 195 °C, hold for 8 min, ramp to 270 at 15 °C min-1 and hold at 270 °C for 2 min. FAME peaks were integrated using the HP Chemstation software package and individual FAMES were identified by comparison of retention times to standards: GLC-84 (Nu Chek Prep USA) as well as individual standards of stearidonic, eicosapentaenoic and arachidonic acid methyl esters (Sigma–Aldrich, MO, USA). FAMES from all samples were quantified using their peak areas compared to the peak area of the C19:0 internal standards.

### 2.1.9. Data analyses

Quantitative values were expressed as treatment means ± standard error. Prior to analysis, percentage data were arcsine transformed. To meet the assumptions of ANOVA, all data were analyzed using Levene’s homogeneity of variance test prior to a two-way ANOVA. If the data violated the assumptions of the two-way ANOVA, a Kruskal–Wallis test was run to compare the independent effects of enrichment and feed frequency. The effects of rotifer enrichment, feeding frequency and their interaction on growth, survival, and stress resistance (SAI) were analyzed using a two-way ANOVA. When no interactive effects were observed, rotifer enrichment treatments were combined across feeding frequencies and feeding frequency treatments were combined across enrichment treatments. A Tukey–Kramer honestly significant difference (HSD) test was used for multiple comparisons among means (Kramer, 1956). All statistical analyses
were performed using SAS statistical software (version 9.1.3, SAS Institute, Cary, North Carolina, USA) and a P-value \( \leq 0.05 \) was considered significant.

2.2. Experiment 2: comparison of Artemia enrichment media and feeding frequency

2.2.1. Experimental system

For Experiment 1.2, larvae were initially reared from hatching through 14 dph in 300-L larval rearing tanks (LRTs) supported by a recirculating system as described below. Yolk sac stage larvae were stocked at a density of 50 larvae/L at 34 salinity and at 22 °C, and larvae were reared on a standard feeding regime (Morris et al., 2008; Ostrowski et al., 2011) until the start of the experiment. During the pre-experimental period, larvae were fed rotifers cultured on RotiGrow and then enriched with N-Rich (Reed Mariculture Inc., Campbell, CA) before feeding to the larvae.

Experimental units consisted of gray, 30-L cylindrical (diam. = 38 cm, depth = 40 cm) mini larval rearing tanks (mini LRTs) with center draining standpipes and in-flow manifolds. The mini LRTs were supported by a common recirculating system, including a 0.11-m³ bubble bead filter (Aquaculture Systems Technology, New Orleans, LA); a 40-W UV sterilizer (Emperor Aquatics, Pottstown, PA); a 1-HP heat pump, a foam fractionator (Top Fathom TF300A, 1/10 HP Foam Fractionator Pump) (Orangeburg, NY); ¾ HP pump (Jacuzzi Stingray) (Chino Hills, CA); and a 25-μm cartridge filter. Full strength seawater (34 salinity) was recirculated through each tank at an exchange rate of 200% daily, and temperature was held at 22 °C using a heat pump (Aqualogic, San Diego, CA). Air was supplied to each tank by two air stones on either side of the center standpipe at 90 mL/min. Illumination was provided at 500 lx at the water surface by two 40-W full spectrum fluorescent light bulbs suspended over the tanks. Photoperiod was 18L:6D starting at 0600 h and ending at 2200 h.

2.2.2. Experimental design

Larvae were stocked into 12 mini LRTs at a density of 8 larvae/L at 34 salinity and 22 °C. Larvae were fed newly-hatched unenriched Artemia franciscana Instar 1 nauplii (Argent Laboratories, Redmond, WA, USA) from 15 to 20 dph. To study the effects of Artemia enrichment on larval performance, larvae were fed, beginning 18 dph and continuing through 32 dph, Artemia Instar II metanauplii enriched with two different treatment media: Algamac 3000 and DC DHA Selco (15 mg/g dw EPA, 30 mg/g dw DHA, 50 mg/g dw sum n-3 HUFAs, INVE Aquaculture, Belgium). In addition, larvae were fed unenriched Artemia Instar II metanauplii as a control treatment. Four replicate tanks were maintained per treatment. Enriched Artemia concentration was 0.5/mL on 18 dph and then increased daily to 3.0/mL by 23 dph and continuing through the beginning of the metamorphic period approximately 32 dph. Artemia were fed twice daily (0830 and 1600 h) to the targeted level. After the first (0830 h) feeding, enriched Artemia were held at 10 °C to maintain nutritional profile before the second (1600 h) feeding.

2.2.3. Experimental conditions and larval growth and survival

Experimental conditions and water quality were maintained as described for Experiment 1 above. Larval growth was determined using volumetric methods on 14, 21, 25 and 33 dph as described for Experiment 1 above. Survival was determined at terminal sampling on 33 dph.

2.2.4. Fatty acid composition (Experiment 1.2)

Fatty acid composition of the enriched Artemia and larval whole bodies from each treatment at the beginning and at the end of the experiment were determined as described above. Fatty acid profiles are summarized in the results section.

2.2.5. Data analyses

Data analyses followed those described for Experiment 1 above. However, in Experiment 2, the effects of Artemia enrichment on growth, survival and larval fatty acid composition were analyzed using a one-way ANOVA and significant differences among treatments were tested by Tukey–Kramer HSD test.

2.3. Experiment 3: minimize reliance on live prey through artificial microdiets (MDs) for co-feeding and early weaning

2.3.1. Experimental system and animals

Experiment 3 was conducted to test the performance of red porgy larvae on different microdiets. For Experiment 3, larvae were initially reared from hatching through 15 dph in 300-L LRTs supported by a recirculating system as described above for Experiment 1. Embryos were stocked into three 300-L LRTs at a density of approximately 39 embryos per L (11,906 per tank) at 34 salinity and at 22 °C, and larvae were reared on a standard feeding regime (Morris et al., 2008; Ostrowski et al., 2011) until the start of the experiment. During the pre-experimental period, larvae were fed rotifers cultured on RotiGrow and then enriched with N-Rich (Reed Mariculture Inc., Campbell, CA) before feeding to the larvae.

The experimental units consisted of gray, 30-L cylindrical (diam. = 38 cm, depth = 40 cm) mini larval rearing tanks with center draining standpipes and in-flow manifolds supported by a recirculating system as described in Experiment 1.2 above. Full strength seawater (34 salinity) was recirculated through each tank at an exchange rate of 200% daily and was held at 22 °C. Air was supplied to each tank by two air stones on either side of the center standpipe at 90 mL/min. Illumination was 500 lx at the water surface, and photoperiod was 18L:6D starting at 0800 h and ending at 0200 h.

2.3.2. Experimental design

On 16 dph, red porgy larvae were stocked into twenty-four 30-L mini LRTs at an initial density of 6.2 fish/L, but possibly due to transfer stress, many fish succumbed overnight, leaving a starting experimental density of 3.9 fish/L. To compare the performance of red porgy larvae on different microdiets, fish were co-fed Artemia and one of the following three microdiets: (1) Gemma Micro (Skretting, Nutreco, Canada), (2) a UNCW-formulated zein-bound microdiet (MBD) and (3) Otohime (Reed Mariculture Inc., Campbell, CA), and (Table 1). Fish were reared according to UNCW’s larviculture protocol for red porgy (Morris et al., 2008; Ostrowski et al., 2011) (Table 1). Fish were co-fed live feed two times per day (0830 and 1600 h) and microdiets (1–6 mg/fish/day) four times per day (0830, 1130, 1430, and 1630 h) as reported for other marine fish larvae (Teshima et al., 2004). UNCW MBD and the commercial microdiets (MDs) were fed using a small spoon and blowing the feed into the water. Microdiet particle sizes were adjusted according to fish mouth sizes based on visual observation.

UNCW zein-microbound diet (MBD) was formulated on the basis of previous studies on southern flounder Paralichthys lethostigma (Table 2) (Alam et al., 2013) and nutrient requirement information for other marine fish larvae (Teshima et al., 2004). The UNCW MBD contained 58% crude protein and 16% lipid and was formulated with different high quality protein sources, including menhaden fish meal, soybean protein concentrate, squid meal and krill meal. The UNCW MBD also contained 1% attractants (alanine, glycine, taurine and betaine), 0.5% methionine, 0.5% lysine, 5% vitamin and 5% minerals. Menhaden fish oil and soybean lecithin were added as lipid sources (Table 2).

Before preparing the MBD, all protein sources were sieved (mesh size 125–250 μm). The MBD was prepared according to Teshima et al. (1982) and Kanazawa et al. (1989) and then ground and sieved into particles ranging from 125 to 550 μm. The lipids
Table 1
Protocol used to feed live feed and commercial microdiets (MDs) and the UNCW microbound diet (MBD) to larval red pony.

<table>
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<tr>
<th>1</th>
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<th>15</th>
<th>20</th>
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<th>31</th>
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<tr>
<td>Rotifers (5–20/mL)</td>
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<td>Feeding of live feed</td>
<td>2 times/day (0830 and 1600 h)</td>
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<tr>
<td>Feeding rate of MFS/MBD</td>
<td>2 times/day (0830 and 1600 h)</td>
<td>Daniels and Watanabe (2003), Morris et al. (2008), Ostrowski et al. (2011)</td>
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<tr>
<td>Feeding frequency of MDs/MBD</td>
<td>4 times/day (0830, 1130, 1430 and 1630 h)</td>
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Artemia (0.5–3/mL)

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<th>MDs/MBD (125–250 μm)</th>
<th>MDs/MBD (250–355 μm)</th>
<th>MDs/MBD (355–500 μm)</th>
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<tr>
<td>2.3.3. Composition of University of North Carolina Wilmington microbound diet.</td>
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</table>

<table>
<thead>
<tr>
<th>Ingredients</th>
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<tr>
<td>Soy protein concentrate</td>
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<tr>
<td>Krill meal</td>
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<tr>
<td>Squid meal</td>
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<td>Menhaden meal</td>
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<td>Zein</td>
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<td>Lipid%</td>
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<td>Energy kJ/g diet</td>
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</table>

The larvae were collected, rinsed with freshwater and blotted dry and wet weights (mg/fish) were measured by weighing fish with an electronic microbalance. The larvae were freeze dried and dry weights were measured using a microbalance and then stored at −80 °C for proximate analysis.

2.3.5. Larval proximate and fatty acid composition

Proximate analysis followed the method of AOAC (2000). Fatty acid composition of the larval whole bodies was determined as described above.

2.3.6. Data analyses

Data analyses followed those described for Experiment 1 above. Growth data were analyzed by one-way ANOVA (JMP ver. 7, SAS, USA). A Tukey–Kramer HSD test was used for multiple comparisons among means (Kramer, 1956).

3. Results

3.1. Experiment 1: comparisons of rotifer enrichment media and feeding frequency

3.1.1. Growth and survival

There were no significant (P > 0.05) main or interactive effects of rotifer enrichment media and feeding frequency on larval NL (Table 3), dry weight (Table 4) or survival on any sampling day for the duration of the study. Initial (0 dph) NL under both feeding frequencies was 2.52 mm and increased steadily to 5.14 mm on 18 dph (Table 3). Initial (0 dph) dry weight under both feeding frequencies was 0.02 mg among treatments and increased steadily to 0.35 mg on 18 dph (Table 4). Survival under both feeding frequencies, fell sharply to 42.3 ± 5.2% by 6 dph, and then more gradually to 29.8 ± 3.3% on 13 dph and to 22.8 ± 3.5% on 18 dph.

3.1.2. Hyposaline stress resistance (SAI)

There were no significant main or interactive effects of larval enrichment media and feeding frequency on larval SAI at any sampling period for the duration of the study (Fig. 1). Under both feeding frequencies, mean SAI values among enrichment treatments were 39.4, 208.1, 627.2 and 939.3 after 12, 24, 36 and 48 h, respectively (Fig. 1). From 24 h post-transfer onward, however, mean SAI values were consistently highest for larvae fed rotifers enriched with Algamac-ARA followed by Algamac 3000 and then protein Selco treatment and were lowest for Rotifer Diet (Fig. 1A). Under all enrichment treatments, mean SAI values were consistently higher for larvae fed 2×/day than for larvae fed 1×/day (Fig. 1B). After 48 h of the hyposaline challenge, a highly significant positive correlation (r = 0.99, P < 0.01, N = 4) between larval SAI and rotifer DHA concentration was evident.

3.1.3. Fatty acid composition of enriched rotifers

Lipid and fatty acid composition of enriched rotifers are shown in Table 5. Values are presented for rotifers sampled before the morning (0800 h) feeding and after the enriched

sources (menhaden fish oil and soybean lecithin) were mixed with 300 mL of 60% ethanol in an electric blender. The zein and the remaining ingredients were added, mixed thoroughly until all the ethanol evaporated. The resulting dough was freeze dried for 48 h or more as necessary. Dry diets were sorted with sieves (125, 250, and 355 μm mesh sizes) to obtain the desired sizes of diets after removal of ethanol by freeze-drying and crumbling into particles. The MBD was stored at −24 °C until used.

2.3.3. Water quality parameters

Total ammonia nitrogen (TAN) (mg/L) was monitored weekly (HACH DR 850, Loveland, CO, USA). Temperature, salinity, dissolved oxygen and pH (YSI 55, Yellow Springs, OH, USA) of the water were monitored every two days. The ranges of water quality parameters were maintained at optimal levels (Morris et al. 2008) as follows: temperature 19.2–21.7 °C, pH 8.2–8.4, salinity 33.1–34.2, dissolved oxygen 7.3–8.6 mg/L, TAN 0.1–0.3 mg/L, and nitrite 0.09–0.14 mg/L. Light intensity (470–580 lx) at the water surface of each tank was measured with a light meter (Extech Instruments, Waltham, MA, USA). Airflow (40–60 mL/min) to each tank was monitored using a flow meter (Cole-Parmer Instrument, Vernon Hills, IL, USA) and adjusted as needed. Tank surfaces were skinned daily with paper towels to remove oil films.

2.3.4. Growth performance, survival determination, and sample collection

The fish were sampled initially and on the final day (day 16) of the experiment to minimize handling stress. Survival (% from initial stocking) was monitored on day 7, 12, and 16 (final sampling). Performance of fish on each test microdiet was assessed by final body weight (FBW), total length (TL), and survival (% survival of initial test fish). Measurements of TL were conducted using a microscope with 10 fish randomly collected from each tank. For body weight, all
rotifers were held at 10 °C for 8 h (i.e., "cold-banked") before the afternoon (1600 h) feeding. Since duplicate determinations were made for each fatty acid, no statistical comparisons were made. In general, afternoon values were similar to or slightly below the morning values. Total lipid was similar among treatments (mean = 361.2, range = 342.3–376.1 mg/g dry wt.) (Table 5). Total SFA appeared to be higher in rotifers fed Algamac (47.34–48.39% TFA) than in the other treatments (36.48–37.56% TFA). Total MUFA appeared to be higher in the Rotifer Diet and DHA Protein Selco treatments (31.67–32.61% TFA) than in the Algamac + ARA treat-
Fig. 1. Survival Activity Index (SAI) of larval red porgy under different rotifer enrichment (A) and feeding frequency (times/day) treatments (B). Data represent means (±SEM). In A, data were combined across both feeding frequencies (N = 8). In B, data were combined across all enrichment treatments (N = 16). No significant differences in SAI among treatments were observed.

ment (27.07–27.65% TFA) and lowest in the Algamac treatment (22.62–23.98% TFA).

Total n-3 PUFA levels appeared to be higher in rotifers fed DHA Protein Selco (9.12–10.26% TFA) and Algamac 3000 + ARA (9.84–11.00% TFA) than in rotifers fed Rotifer Diet (5.91–6.78% TFA) or Algamac 3000 (6.51–6.65% TFA) (Table 5). Among the n-3 PUFAs, concentration of EPA 20:5n-3 appeared to be lower in rotifers enriched with Algamac 3000 (2.66–2.79% TFA) than in the other treatments (3.79–5.52% TFA). DPA docosapentaenoic acid 22:5n-3 ranged from 1.10 to 1.90% TFA among treatments. DHA 22:6n-3 was undetected in rotifers fed Rotifer Diet, but ranged from 1.30 to 2.02% in rotifers fed DHA Protein Selco and Algamac and appeared to be highest in the Algamac + ARA treatment (2.40–2.76% TFA).

Total n-6 PUFA levels of rotifers appeared to be lowest in the Algamac treatment (2.27–2.79% TFA), intermediate in the Rotifer Diet (3.63–4.74% TFA) and Algamac + ARA (4.54–4.89% TFA) treatments and highest in the DHA Protein Selco treatment (8.00–8.47% TFA) (Table 5). Among the n-6 PUFAs, rotifers enriched with
Algamac 3000 + ARA appeared to have higher ARA concentrations (1.46–1.56% TFA) than the other treatments (0.44–1.04% TFA).

3.2. Experiment 2: comparisons of Artemia enrichment media

3.2.1. Growth and survival

From 14 to 21 dph, growth (NL and dry weight) of larvae in the control treatment fed unenriched Artemia was indistinguishable from growth of larvae fed Artemia enriched with Algamac 3000 and DC DHA Selco (Fig. 2). However, a clear departure in growth was observed from 25 dph, when larvae fed enriched Artemia showed markedly improved growth. Larval NL on 14 dph was 4.83 mm among treatments, with no significant differences (Fig. 2A). Mean NL remained similar among treatments through 21 dph (6.64 mm) and 25 dph (7.64 mm) (Fig. 2A). By 33 dph, however, there were highly significant (P < 0.001) differences among treatments, with the Algamac 3000 (12.15 mm) and DC DHA Selco (11.68 mm) treatments producing higher larval NL than the control treatment (7.85 mm), but not significantly different between enrichment treatments (Fig. 2). Larval dry weight on 14 dph was 0.2 mg in all treatments, with no significant differences (Fig. 2B). Dry weight remained similar in all treatments through 21 dph (1.18 mg) (Fig. 2B). By 25 dph, however, there were highly significant (P < 0.01) differences among treatments, with the DC DHA Selco (3.08 mg) and Algamac 3000 (2.80 mg) treatments producing higher dry weight than the control treatment (2.0 mg), but with no differences between enrichment treatments (Fig. 2B). On 33 dph, the Algamac 3000 (11.3 mg) and the DC DHA Selco (9.17 mg) treatments were significantly larger than the control (2.18 mg) treatment, but with no differences between enrichment treatments (Fig. 2B). On 33 dph, survival (mean ± SEM, N = 4) was markedly higher in the DC DHA Selco treatment (54.61 ± 6.43%) than in the Algamac 3000 treatment (36.7 ± 4.03%) and was lowest in the control treatment (5.23 ± 2.58%).

3.2.2. Fatty acid composition of enriched Artemia

Lipid and fatty acid composition of enriched Artemia are shown in Table 6. Values are presented for Artemia sampled before the morning (0800 h) feeding and after the enriched Artemia were held at 10°C for 8 h (i.e., “coldbanked”) before the afternoon (1600 h) feeding. No significant (P > 0.05) differences between morning and afternoon concentrations were observed for any fatty acid. No significant treatment differences were observed in total lipid (290 mg/g dry wt.) or in MUFA (41.9% TFA) (Table 6). Total SFA was lowest in the DC DHA Selco (27.5% TFA) and the unenriched (control) (29.8%) highest (P < 0.05) in the Algamac 3000 (33.08% TFA) treatments.

No significant differences were observed in total n-3 PUFA levels among Artemia enriched with different treatments (21.3% TFA) (Table 6). Among the n-3 PUFAs, concentration of stearidonic acid (STD) 18:4n-3 was higher (P < 0.05) in the control (3.79% TFA) and Algamac enriched Artemia (3.22% TFA) compared to Artemia enriched with DC DHA Selco (2.14% TFA). There were no significant differences in EPA 20:5n-3 (2.53% TFA) among treatments. Concentration of DPA docosapentaenoic acid 22:5n-3 averaged (1.45% TFA) in DC DHA Selco and Algamac-enriched Artemia, but was not detected in the control Artemia. DHA 22:6n-3 levels in Artemia fed DC DHA Selco and Algamac (2.40% TFA), although considerably lower than those provided in their diets (Algamac = 41% DHA, DC DHA Selco = 30% DHA), were significantly (P < 0.05) higher than those found in unenriched control Artemia (0.66% TFA) (Table 6). No significant treatment differences were observed in total n-6 PUFA levels of Artemia (6.62% TFA) (Table 6). Among the n-6 PUFAs, concentration of ARA was higher (P < 0.05) in Artemia enriched with Algamac 3000 (2.17% TFA) than with DC DHA Selco (0.32% TFA) and was lowest in the unenriched controls (0.29% TFA).

3.2.3. Larval fatty acid composition

Whole body lipid and fatty acid composition of red porgy larvae on 13 dph (initial) and on 25 dph (final) are shown in Table 7. On 13 dph, mean total lipid level among treatments was 306 mg/g dry wt. Among the n-3 PUFAs, DHA averaged 11.8% TFA, EPA 8.01%, and ALA 12.9%. Among the n-6 PUFAs, ARA averaged 4.45% TFA, and LA linoleic (18:2n-6) 5.42%.

On 25 dph, no significant differences among treatments in total lipid (170 mg/g dry wt.) were observed. On 25 dph, concentration of SFA was higher (P < 0.05) in larvae fed the unenriched control Artemia (30.6% TFA) than in larvae fed prey enriched with Algamac 3000 (27.8% TFA) and was lowest in larvae fed prey enriched with DC DHA Selco (25.7% TFA). Total SFA was higher (P < 0.05) in 13 dph larvae (30.3% TFA) compared to 25 dph larvae fed prey enriched with DC DHA Selco (25.7% TFA). On 25 dph, no significant differences among treatments were observed in total MUFA (33.7% TFA).

On 25 dph, no significant differences among treatments were observed in total n-3 PUFA levels (25.5% TFA) (Table 7). Among the n-3 PUFAs, EPA was higher (P < 0.05) in larvae fed Artemia enriched with DC DHA Selco (8.02% TFA) than in larvae fed Artemia enriched with Algamac (7.24% TFA), or fed unenriched Artemia (6.82% TFA). On 25 dph, EPA was higher (P < 0.05) in the DC DHA Selco treatment (8.02% TFA) than in the Algamac (7.24%) or control (6.92% TFA) treatments. On 25 dph, DHA levels were higher (P < 0.05) in larvae fed Artemia enriched with DC DHA Selco (10.3% TFA) than in larvae fed Artemia enriched with Algamac (7.09% TFA) and was lowest (P < 0.05) in larvae fed unenriched Artemia (3.55% TFA) (Table 7).

The total n-6 PUFA concentration was higher (P < 0.05) in larvae fed Artemia enriched with Algamac (13.6% TFA) than in larvae fed Artemia enriched with DC DHA Selco (11.0% TFA) or unenriched Artemia (10.2% TFA) (Table 7). Among the n-6 PUFA, concentration of ARA was higher (P < 0.05) in larvae fed Artemia enriched with Algamac (5.42% TFA) than in larvae fed DC DHA Selco-enriched (4.10% TFA), or unenriched Artemia (4.63% TFA) (Table 7). ARA concentration in larvae enriched with Algamac was higher than those found on 13 dph (4.45% TFA).

Whole body lipid and fatty acid composition of red porgy larvae on 33 dph are shown in Table 8. No significant differences were observed in total lipid (148 mg/g dry wt.), SFA (27.0% TFA), or MUFA (30.6% TFA) among treatments. On 33 dph, no significant differences among treatments were observed in total n-3 PUFA levels (28.6% TFA). Among the n-3 PUFAs, EPA was higher in control larvae fed unenriched Artemia (7.63% TFA) than in larvae fed Artemia enriched with DC DHA Selco (6.23% TFA) or with Algamac 3000 (5.79% TFA). Concentration of DHA was higher (P < 0.05) in larvae fed Artemia enriched with DC DHA Selco (10.6% TFA) than in larvae fed unenriched Artemia (6.40% TFA).

On 33 dph, total n-6 PUFA concentration was highest (P < 0.05) in larvae fed unenriched Artemia (13.7% TFA) compared to larvae fed Artemia enriched with Algamac (12.5% TFA), or with DC DHA Selco (10.5% TFA) (Table 8). Among the n-6 PUFAs, concentration of 18:2n-6 linoleic acid (LA) was highest (P < 0.05) in larvae fed the unenriched Artemia (7.03% TFA) compared to larvae fed Artemia enriched with Algamac (4.73% TFA). Concentration of ARA was higher (P < 0.05) in larvae fed the unenriched Artemia (6.66% TFA) than in larvae fed Artemia enriched with DC DHA Selco (3.42% TFA) and Algamac (4.52% TFA). Concentration of 22:5n-6 docosapentaenoic acid (DPA) was higher (P < 0.05) in larvae fed Artemia enriched with Algamac (3.28% TFA) than in larvae fed Artemia enriched with DC DHA Selco (0.85% TFA), or in unenriched Artemia (undetected).
3.3. Experiment 3: artificial microdiets (MDs) for co-feeding and early weaning

The mean initial total length of the red porgy larvae was 5.4 mm. Otohime produced a final mean length of 14.4 ± 0.4 mm, followed by Gemma at 13.7 ± 0.5 mm and UNCW at 13.5 ± 0.4 mm (Table 9). Mean initial wet weight of red porgy larvae was 1.54 mg. Otohime produced a mean wet weight per fish of 53.65 ± 5.28 mg, followed by Gemma at 47.06 ± 5.06 mg and UNCW at 46.11 ± 4.17 mg (Table 9). No significant differences were found in final total length or wet weight among treatments after the 16-day feeding trial (Table 9). No significant differences in survival were observed among larvae fed the different microdiets on days 7, 12 and 16 of the feeding trial (Table 9). Final survival (N = 3) after 16 days was 39 ± 9.45 ± 8 and 43 ± 11% for fish fed Gemma, UNCW and Otohime MDs, respectively.

After the feeding trial, crude protein (range = 63.0 ± 0.58 to 64.6 ± 0.67% dry basis, N = 3) and total lipid contents (range = 15.0 ± 1.2 to 16.0 ± 2.0% dry basis) in red porgy larval whole bodies fed different microdiets were not significantly different among treatments. Fatty acid composition of the red porgy larval fed the different microdiets are presented in Table 10. Linoleic acid
(18-2n-6) was significantly higher (P < 0.05) in larvae fed Gemma Micro than in Otohime, but there were no significant differences between Gemma Micro and UNCW MBD. However, EPA (20:5n-3) content in larvae fed Otohime was significantly higher than in larvae fed Gemma. DHA (22:6n-3), and total n-3PUFA content in fish larvae were not significantly different among the treatments. However, total n-6PUFAs were significantly higher in larvae fed the Gemma micro than the larvae fed Otohime (Table 10). Total PUFAs were not significantly different between the larvae fed Gemma Micro and UNCW MBD.

### 4. Discussion

#### 4.1. Experiment 1: comparisons of rotifer enrichment media and feeding frequency

Only modest levels of rotifer enrichment were achieved in all treatments, with post-enrichment essential polyunsaturated fatty acid (PUFA) levels in rotifers well below those found in their respective media. Despite generally low enrichment levels across treatments, the relative PUFA levels in enriched rotifers suggested...
<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>33dph</th>
<th>Control</th>
<th>DC DHA Selco</th>
<th>Algalam 3000</th>
</tr>
</thead>
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<tr>
<td>16:0</td>
<td>13.3 ± 0.12</td>
<td>13.5 ± 0.63</td>
<td>14.3 ± 0.04</td>
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<tr>
<td>16:1-7</td>
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<td>17:0</td>
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<td>2.09 ± 0.05</td>
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<td>20:0</td>
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<td>20:1</td>
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<td>20:5n-3</td>
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<td>22:5n-3</td>
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<td>∑ SFA</td>
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<td>∑ MUFA</td>
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<td>Total lipid mg/g</td>
<td>152 ± 23.8</td>
<td>126 ± 13.9</td>
<td>167 ± 25.4</td>
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Fatty acids in trace amounts (<0.5%) are not reported.

Means not sharing a common letter are significantly (P<0.05) different.
Total lipid is reported in mg/g dry weight.

**Table 8**

<table>
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<th>Parameter</th>
<th>Feeding period (day)</th>
<th>Gemma</th>
<th>UNCW</th>
<th>Micrdiet</th>
<th>Otohime</th>
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<tr>
<td>Survival (%)</td>
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<td>Body weight (mg)</td>
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<td>46.11 ± 4.17</td>
<td>53.65 ± 5.28</td>
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**Table 9**

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<tr>
<th>Fatty acids</th>
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<th>UNCW</th>
<th>Otohime</th>
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<td>2.86 ± 0.25</td>
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<td>12.7 ± 0.29</td>
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<td>13.6 ± 0.27</td>
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Table 10

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<th>Fatty acids</th>
<th>Gemma</th>
<th>UNCW</th>
<th>Otohime</th>
</tr>
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treatments and indicated that subtle differences in stress resistance were manifested even when no obvious differences in larval growth and survival were evident (Ako et al., 1994). Highest mean SAI values were found in larvae fed ARA-supplemented rotifers (i.e., the Algalac + ARA treatment). This is similar to what Koven et al. (2001b) found in gilthead seabream, where ARA supplementation to rotifers did not affect larval growth or survival to the end of the rotifer feeding period (19 dph), but resistance of these larvae to handling stress was improved. In black sea bass larvae, dietary supplementation of ARA within the range of 0–6% improved growth and survival from the first feeding through metamorphic stages (Rezek et al., 2010), and supplementation of ARA within the range of 6–12% promoted the adaptive physiological responses to hypoxia (Carrier et al., 2011). Based on the results of the present study, bioencapsulation of rotifers with a combination of enrichment products that supplemented both DHA and ARA provided red porgy larvae with the highest stress resistance and is recommended among the treatments tested. Work is needed to improve uptake of enrichment media by rotifers to further elevate PUFA concentration over the modest levels attained in this study.

4.2. Comparisons of Artemia enrichment media

Among the n-3 PUFAs, DHA 22:6n-3 levels in Artemia fed DC DHA Selco and Algalac were higher than those found in unenriched control Artemia, reflecting relative DHA levels found in these enrichment products (Algalac = 41% DHA, DC DHA Selco = 30% DHA, N. oculata = 0% DHA). Despite this, levels of DHA enrichment attained in Artemia were much lower than those provided in their diets. Artemia enrichment is difficult due to their rapid development and catabolism of DHA (Sorgeleos et al., 2001). It is possible that higher levels of DHA in Artemia could be achieved if the enrichment period is extended from 16 h (as used in this study) to 19–24 h (McEvoy et al., 1995). Enrichment success in Artemia also depends on genetic characteristics. Chinese strains of Artemia accumulated higher levels of DHA than the Great Salt Lake strain or the San Francisco Bay strain used in this study (Evjemo et al., 1997). Among the n-6 PUFA concentrations of ARA was higher in Artemia enriched with Algalac than with DC DHA Selco and was lowest in the unenriched controls. The presence of ARA in Artemia from all three treatments, although lacking in the enrichment media, is likely attributable to endogenous ARA (0.8% TFA) in Artemia (Couetateau and Mourente, 1997).

Whereas no differences in larval growth or survival were evident through the rotifer feeding phase on 18 dph, distinct treatment effects on larval performance emerged during the Artemia feeding phase. Growth and survival among larvae fed enriched or unenriched Artemia remained similar through 21 dph; however, from 25 to 33 dph, larvae fed Artemia enriched with Algalac and DC DHA Selco were markedly larger (Fig. 2) and showed much higher survival than control larvae fed unenriched Artemia. Superior performance of larvae on Artemia enriched with Algalac and DC DHA Selco is consistent with the higher levels of DHA provided by these enrichment products. Clearly, providing sufficient dietary n-3 PUFA (especially DHA) through bioencapsulation of Artemia is crucial to normal growth and survival of larval red porgy. This is in accord with what was reported in red sea bream Pagrus major where larvae fed rotifers enriched with high levels of DHA (≥20%) grew faster than those fed rotifers enriched with ≤2% DHA (Watanabe et al., 1989). Improved growth with higher dietary DHA has also been reported in larval yellowtail Seriola quinquemaculata (Furuita et al., 1996; Copeman et al., 2002), yellowtail snapper Ocyurus chrysurus (Faulk and Holt, 2005), cobia Rachycentron canadum (Faulk et al., 2005), and black sea bass Centropristis striata (Rezek et al., 2010).

The lack of differences in growth and survival related to DHA-enrichment of rotifers and the clear differences observed in response to DHA-enrichment of Artemia seem contradictory. However, significant differences in response to Artemia enrichment were not evident until 25 dph after 11 days of feeding. It is possible, therefore, that the 15-day feeding period of enriched rotifers was insufficient for treatment effects to be demonstrated and that differences in growth rates were accentuated during the Artemia phase when larval feeding and specific growth rates were much higher. Andrade et al. (2012) reported that in larval red porgy, live feed demand increased sharply at about 5.6 mm TL (Andrade et al., 2012), a body length reached around 18 dph in the present study (Fig. 2) when the rotifer feeding Experiment 1.1 was completed. On 25 dph and on 33 dph, levels of essential n-3 PUFAs EPA and DHA were generally higher in tissues of larvae fed Artemia enriched with DC DHA Selco or Algalac than in larvae fed unenriched Artemia. This is consistent with the higher dietary levels of DHA provided in these treatments and the importance of DHA to larval development.

4.3. Experiment 2: artificial microdiets for co-feeding and early weaning

Total replacement of live food with microdiets has been accomplished in shrimp, but not in marine fish (Cahu and Zambonino-Infante, 2001). Co-feeding live and inert diets has been found to produce higher growth and survival than feeding either live feeds or microdiets alone (Kolkovski et al., 1995; Wang et al., 2005). In red porgy larvae, direct weaning from rotifers to dry diet resulted in significantly lower growth, survival, pancreatic (trypsin and lipase), and intestinal (alkaline phosphatase) enzyme-specific activity, compared to feeding regimes using Artemia as an intermediate step (Andrade et al., 2012). ‘Co-feeding’ weaning protocols applied in the present experiment simultaneously used inert and live diets to allow a fast and efficient change-over period onto dry microdiets from live feed (Koven et al., 2001a). Zein bound MBDs were selected as experimental diets in this study because they were used successfully in the larval rearing of red sea bream and Japanese flounder (Kanazawa et al., 1989; Teshima et al., 2004). Quality MBD for larval and juvenile aquatic animals are needed to meet the following criteria: inclusion of nutritionally well-balanced nutrients, high water-stability (minimal leaching of nutrients), high digestibility, and proper suspension in a water column (Bengtson, 1993; Teshima et al., 2000; Izquierdo and Fernandez-Palacios, 2001). In this experiment, larvae fed UNCW MBD containing both fish meal and squid meal as protein sources showed comparable growth performance to those fed the commercial microdiets Gemma and Otho-Rome after 16 days of feeding. Fish meal and squid powder were found to have a high nutritional value as protein sources for L. calcarifer larvae by virtue of a synergistically favorable amino acid profile and moderate to high digestibility (Nankervis and Southgate, 2006; Saenz de Rodriguez et al., 2011).

In this study, the low survival (39–45%) of red porgy larval co-fed live feed and microdiets could be due to leaching losses of essential soluble nutrients. Rapid rates of loss have been reported for free amino acids supplementing microdiets (Lopez-Alvarado and Kanazawa, 1994; Kvale et al., 2006). Extensive leaching of nitrogenous compounds from microdiets based on intact fish protein sources demonstrated that leaching can also occur for water-soluble macromolecules. At the same time, some leaching of nutrients may be beneficial due to their roles as attractants (Koven et al., 2001a; Kolkovski et al., 2009). A lower availability of the protein in formulated diets compared with live prey is also considered to be an important reason for the low performance of marine fish larvae fed only formulated diets (Kolkovski et al., 2009). However, larvae in this study were fed four times a day to mitigate nutrient deficiencies due to leaching in water. Andrade et al. (2012) recom-
mended feeding of inert diets to red porgy from 30 dph onward, when larvae are physiologically able to digest more complex feed items. In the present study, red porgy larvae were co-fed live feed and microdiets from 16 dph, and limited digestive capacity may have reduced feed utilization and survival in all treatment groups.

In the UNCW-MBD, supplementation of feed attractants likely played an important role in acceptance of dry diets in fish larvae during the weaning period as well as in enhancing growth as reported for other species (Kolkovski et al., 1997; Kolkovski 2000, 2001). The chemo-attractant properties of squid meal were also confirmed for trout fingerlings (Lian et al., 2008) and gilthead sea bream (Kolkovski and Tandler, 2000). The dietary inclusion of taurine produced a significant improvement in the growth of juvenile Japanese flounder (Kim et al., 2005). Carnivorous species usually show a positive feeding response to alkaline and neutral substances, such as glycine, proline, valine, taurine, and betaine (Takaoka et al., 1995).

In general, the fatty acid composition of the fish whole body reflected the composition of their diets (Salih and Bessonart, 2012). The lipid sources of commercial microdiets (Otohime and Gemma) are unknown. However, the n-3 PUFA levels in the larvae fed the UNCW formulated MBD were not significantly different from the two commercial microdiets (Otohime and Gemma) after 16 days of feeding. This was consistent with the similar growth and survival performances observed among treatments. The high DHA content of red porgy larvae reflects the importance of this fatty acid, which is known to be higher in efficiency as an essential fatty acid than EPA (Watanabe and Kiron, 1994). Growth, survival and fatty acid composition of larval whole bodies suggested that red porgy lar- vae were able to utilize UNCW formulated zein-bound microdiets, and that the results were comparable to the commercially available microdiets.

Based on the results of this study, a microbound diet for red porgy larvae has been formulated using a combination of different protein sources and supplemental attractants that produced larval performance (growth, survival and whole body proximate and fatty acid profiles) that were not significantly different from premium commercial microdiets Otohime and Gemma Micro. The success in rearing the larval red porgy (16 dph) with the MBD containing fish meal, soy protein concentrate, squid meal, krill meal and attractants as the major nitrogen sources indicates that red porgy larvae readily assimilate these dietary protein sources for normal growth and development. These results provide a firm basis for developing and testing improved formulations that can potentially yield higher growth, survival and earlier weaning from live prey in red porgy larvae.

5. Conclusions

Red porgy larval growth, survival and stress resistance were improved through bioencapsulation of live prey using commercially available products and proper feeding regimes. During the early feeding stage red porgy larvae showed higher stress resistance when fed rotifers enriched with a strategic combination of enrichment products to boost both DHA and ARA, and when larvae were fed their daily ration allocated over two feedings rather than one. For older larvae, growth and survival were markedly higher in lar- vae fed Artemia metanauplii enriched with DHA. During transition from live prey to artificial feeds, a UNCW-formulated microbound diet produced larval performance comparable to premium, commercial microdiets up to the metamorphic stages. Results define protocols for more effective hatchery production of red porgy juvenile.

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References


