Aquaculture Reports 3 (2016) 93–107

Contents lists available at ScienceDirect

Aquaculture Reports

journal homepage: www.elsevier.com/locate/aqrep

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

Wade O. Watanabe[a,*], Md. Shah Alam[a], Andrew D. Ostrowski[a,1], Frank A. Montgomery[a], Jennifer E. Gabel[a], James A. Morris Jr.[b], Pamela J. Seaton[c]

[a] Center for Marine Science, University of North Carolina Wilmington, USA
[b] Center for Coastal Fisheries and Habitat Research, National Ocean Service (NOS), NOAA, USA
[c] Department of Chemistry and Biochemistry, University of North Carolina Wilmington, USA

ARTICLE INFO

Article history:
Received 31 January 2015
Received in revised form 4 January 2016
Accepted 9 January 2016

Keywords:
Red porgy
Live prey enrichment
Rotifers
Artemia
Microdiets
Microbound diets
Essential fatty acids

ABSTRACT

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 *Nannochloropsis 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.

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.,

Abbreviations: MD, microdiets; MBD, microbound diets; EFAs, essential fatty acids; PVC, polyvinyl chloride; LRTs, larval rearing tanks; 2 dph, 2 days post-hatching; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; ARA, arachidonic acid; DPA, docosapentaenoic acid; ALA, alpha linolenic acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; TFA, total fatty acids; ANOVA, analysis of variance; FBW, final body weight; SAI, survival activity index; FAMEs, fatty acid methyl esters.

* Corresponding author at: University of North Carolina Wilmington, Center for Marine Science, 601 South College Rd., Wilmington, NC 28403-5927, USA. Fax: +1 910 962 2868.
E-mail addresses: watanabew@uncw.edu, wwatanabe@ec.rr.com, watanabe.wo@gmail.com (W.O. Watanabe).
[1] Present address: Center for Coastal Fisheries and Habitat Research, National Marine Fisheries Service, NOAA, USA.

http://dx.doi.org/10.1016/j.aqrep.2016.01.003

1997; Hernandez-Cruz et al., 1999; Roo et al., 1999; Mihelakakis et al., 2001a,b; Papandroulakis et al., 2004; Morris et al., 2008; Roo et al., 2010).

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 enrich 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; Sorgeloos 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, 22-6:n-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-6HUFA arachidonic acid (ARA, 20:4n-6) in live prey can also affect growth or 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 used 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-m^3 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 m (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 16 L:8 D starting at 0700 h and ending at 2300 h. Extraneous light was excluded by black curtains surrounding the water bath.

To begin the experiment, yolksac larvae (0 days post-hatching = 0 dph) 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 dph (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 dw DHA, 10 mg/day dw EPA, 0 ARA; INVE Aquaculture, Belgium), Algamac 3000 (heterotrophically-grown *Schizochytrium* sp.; 41.3% DHA, 14.72% DPA, 1.3% EPA, 0% ARA; BioMarine Aquafauna, CA, USA) and Algamac + ARA (mix of 75% Algamac 3000 and 25% Algamac-arachidonic acid; 2.4% DHA, 0% DPA, 0% EPA, 37% ARA, BioMarine Aquafauna, 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 ± 0.1), 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 under conditions of zero feeding. Using volumetric methods, survival was monitored every 12 h for 48 h. A survival activity index (SAI) to test larvae quality was calculated using the following equation:

$$\mathrm{SAI} = \frac{1}{N}\sum_{i:1}^{k}(N - h_i) \times i$$

where N is the total number of initial larvae, h_i is the cumulative mortality by the ith 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 (BF_3) 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 μm 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 ≤ 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. Yolksac 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-m^3 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 porgy.

1	5	10	15	20	25	31
Rotifers (5–20/mL)						
			Artemia (0.5–3/mL)			
			MDs/MBD (125–250 μm)	MDs/MBD (250–355 μm)	MDs/MBD (355–500 μm)	
Feeding of live feed			2 times/day (0830 and 1600 h) Daniels and Watanabe (2003), Morris et al. (2008), Ostrowski et al. (2011)			
Feeding rate of MFs/MBD			1–6 mg fish/day (Teshima et al. 2004)			
Feeding frequency of MDs/MBD			4 times/day (0830, 1130, 1430 and 1630 h)			

Table 2
Composition of University of North Carolina Wilmington microbound diet.

Ingredients	g/100 g
Soy protein concentrate	8
Krill meal	10
Squid meal	35
Menhaden meal	15
Zein	10
Menhaden fish oil	7
Soybean lecithin	3
Vitamin	5
Mineral	5
Taurine	0.25
Alanine	0.25
Glycine	0.25
Betaine	0.25
Methionine	0.5
Lysine	0.5
Total	100
Protein%	58
Lipid%	16
Energy kj/g diet	19.4

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, Vernor Hills, IL, USA) and adjusted as needed. Tank surfaces were skimmed 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 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 1.1 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 among treatments 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 rotifer 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

Table 3
Growth (notochord length = NL) of larval red porgy under different rotifer enrichment and feeding frequency treatments. Data represent means (±SEM). To compare enrichment treatments, growth data were combined across both feeding frequencies ($N = 8$). To compare feeding frequencies, growth data were combined across all enrichment treatments ($N = 16$). No significant ($P > 0.05$) treatment differences were observed.

Age (dph)	Enrichment			
	Rotifer Diet	DHA Protein Selco	Algamac	Algamac + ARA
0	2.50 ± 0.03	2.53 ± 0.03	2.53 ± 0.03	2.53 ± 0.03
6	3.46 ± 0.11	3.38 ± 0.11	3.28 ± 0.06	3.23 ± 0.12
13	4.33 ± 0.09	4.33 ± 0.08	4.44 ± 0.07	4.35 ± 0.12
18	5.00 ± 0.11	5.18 ± 0.06	5.24 ± 0.11	5.12 ± 0.09

Age (dph)	Feeding frequency (times/day)	
	2×/day	1×/day
6	3.34 ± 0.07	3.34 ± 0.08
13	4.27 ± 0.07	4.45 ± 0.05
18	5.04 ± 0.07	5.22 ± 0.06

Table 4
Growth in dry weight (mg) of larval red porgy under different rotifer enrichment and feeding frequency treatments. Data represent means (±SEM). To compare enrichment treatments, growth data were combined across both feeding frequencies ($N = 8$). To compare feeding frequencies, growth data were combined across all enrichment treatments ($N = 16$). No significant ($P > 0.05$) treatment differences were observed.

Age (dph)	Enrichment			
	Rotifer Diet	DHA Protein Selco	Algamac	Algamac + ARA
0	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
6	0.03 ± 0.00	0.03 ± 0.01	0.03 ± 0.00	0.04 ± 0.01
13	0.13 ± 0.01	0.10 ± 0.02	0.15 ± 0.01	0.13 ± 0.01
18	0.35 ± 0.03	0.37 ± 0.02	0.33 ± 0.02	0.36 ± 0.03

Age (dph)	Feeding frequency (times/day)	
	2×/day	1×/day
0	0.02 ± 0.01	0.02 ± 0.01
6	0.03 ± 0.00	0.03 ± 0.00
13	0.14 ± 0.01	0.12 ± 0.01
18	0.35 ± 0.02	0.36 ± 0.02

Table 5
Total lipid and fatty acid composition (% of total fatty acids) of rotifers enriched with different products (Rotifer Diet, DC DHA Protein Selco, Algamac 3000, Algamac 3000 + ARA). Values represent means ($N = 2$) and are presented for rotifers sampled before the first (0800 h) feeding and after the enriched rotifers were held at 10 °C for 8 h before the second (1600 h) feeding.

Fatty acids	Rotifer treatments							
	Rotifer Diet am	Rotifer Diet pm	DC DHA Selco am	DC DHA Selco pm	Algamac 3000 am	Algamac 3000 pm	Algamac 3000 + ARA am	Algamac 3000 + ARA pm
14:0	4.80	4.77	3.12	3.22	6.27	6.28	5.04	4.79
16:0	26.53	26.47	23.12	24.62	35.70	35.25	27.58	26.76
16:1n-7	16.59	16.29	10.67	11.22	12.14	10.96	13.97	13.60
18:0	5.65	5.24	6.24	6.29	6.42	5.81	4.94	5.39
18:1n-9	7.74	7.57	11.86	12.21	5.93	5.95	6.62	6.46
18:1n-11	5.65	5.52	6.79	6.84	4.32	4.21	5.29	5.24
18:2n-6	3.66	3.19	6.83	6.70	1.99	1.69	3.33	3.08
18:3n-3	0.96	0.93	1.47	1.51	0.73	0.74	0.82	0.80
18:4n-3	Undetected	Undetected	Undetected	Undetected	Undetected	Undetected	Undetected	Undetected
20:1	2.25	1.98	2.35	2.34	1.59	1.50	1.77	1.77
20:2n-6	0.27	Undetected	0.60	0.26	Undetected	Undetected	Undetected	Undetected
20:4n-6	0.81	0.44	1.04	1.04	0.80	0.58	1.56	1.46
20:5n-3	4.64	3.79	5.48	4.60	2.79	2.66	5.52	4.91
22:5n-3	1.18	1.19	1.82	1.71	1.10	1.23	1.90	1.73
22:5n-6	Undetected	Undetected	Undetected	Undetected	Undetected	Undetected	Undetected	Undetected
22:6n-3	Undetected	Undetected	1.49	1.30	1.89	2.02	2.76	2.40
∑SFA	36.98	36.48	32.48	34.13	48.39	47.34	37.56	36.94
∑MUFA	32.23	31.36	31.67	32.61	23.98	22.62	27.65	27.07
∑n-3 PUFA	6.78	5.91	10.26	9.12	6.51	6.65	11.00	9.84
∑n-6 PUFA	4.74	3.63	8.47	8.00	2.79	2.27	4.89	4.54
Total lipid mg/g	349.17	353.81	369.92	342.32	374.69	363.09	376.07	361.20

Fatty acids in trace amounts (<0.5%) are not reported.
Total lipid is reported in mg/g dry weight.

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-

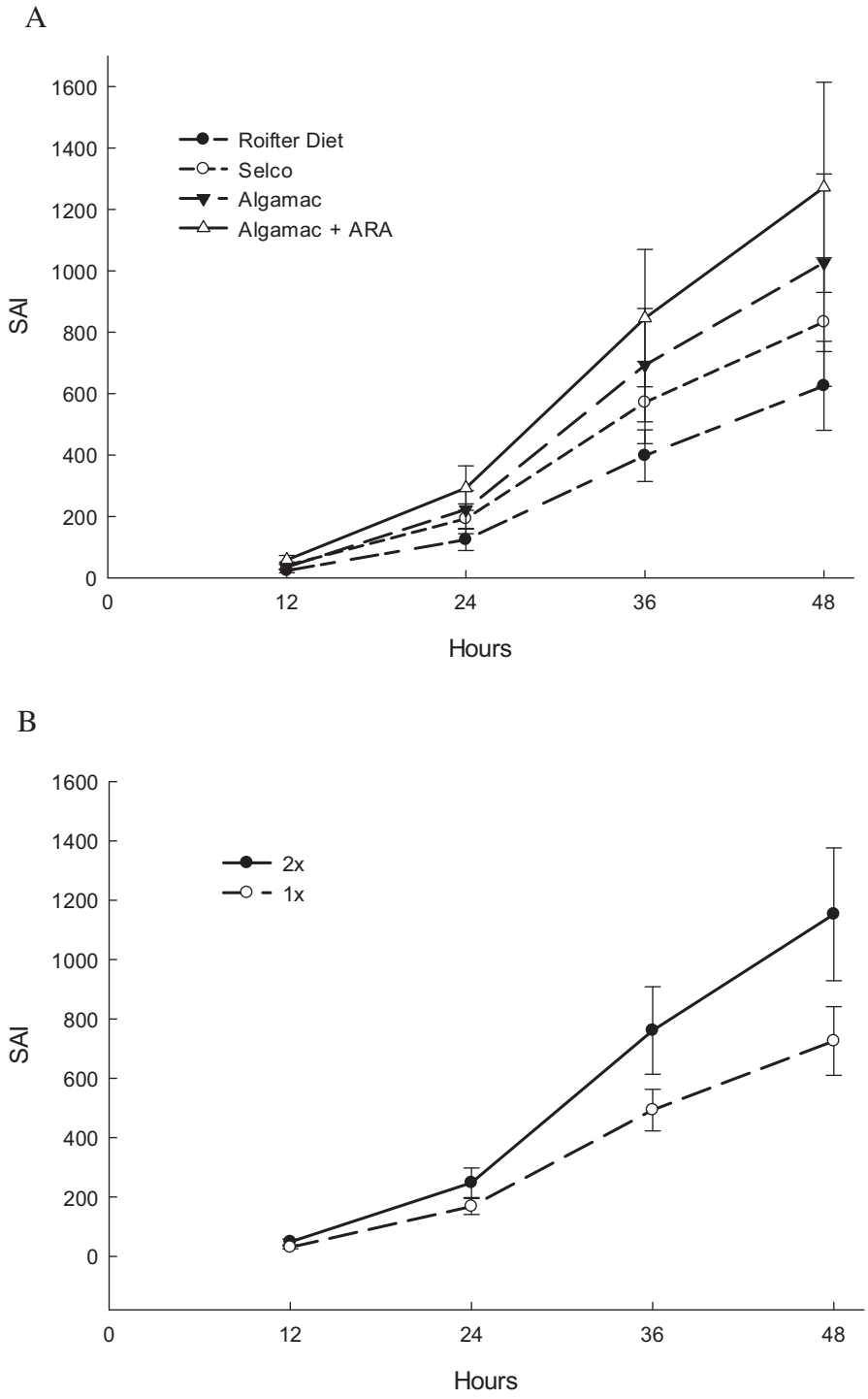

Fig. 1. Survival Activity Index (SAI) of larval red porgy under different rotifer enrichment (A) and feeding frequency (times/day) treatments (B). Data represent means (±SEM). In A, data were combined across both feeding frequencies ($N = 8$). In B, data were combined across all enrichment treatments ($N = 16$). 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 d33 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 MUFAs (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* (6.92% 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 in 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).

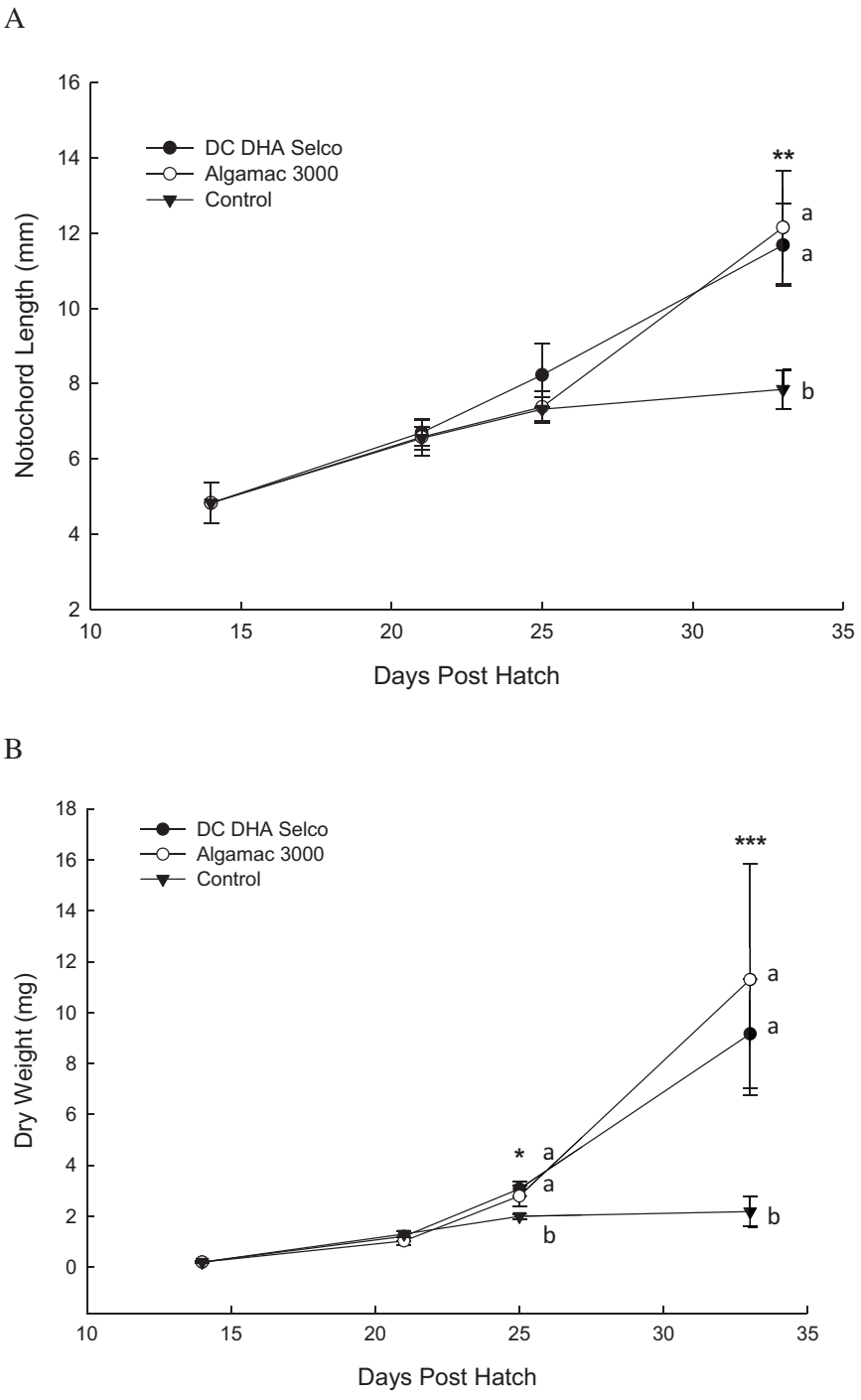

Fig. 2. Growth in notochord length (mm) (A) and in dry weight (mg) (B) of larval red porgy under different *Artemia* enrichment treatments (Experiment 1.2). Data represent means ± SEM ($N = 4$). Asterisks indicate significant treatment differences (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ANOVA). Means not sharing a letter in common are significantly ($P < 0.05$, Tukey–Kramer test) different.

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 larvae fed the different microdiets are presented in Table 10. Linoleic acid

Table 6
Total lipid and fatty acid composition (% of total fatty acids) (mean ± SEM, $N = 3$) of *Artemia* enriched with two different enrichment products, Algamac 3000 and DC DHA Selco, and an unenriched (control) group. Values are presented for *Artemia* sampled before the first (0800 h) feeding and after the enriched rotifers were held at 10 °C for 8 h before the second (1600 h) feeding.

Fatty acids	*Artemia* treatments					
	Control am	Control pm	DC DHA Selco am	DC DHA Selco pm	Algamac 3000 am	Algamac 3000 pm
14:0	2.63 ± 0.19	2.63 ± 0.06	2.94 ± 0.15	2.90 ± 0.17	3.35 ± 0.45	3.37 ± 0.07
16:0	15.2 ± 0.83^{a}	15.2 ± 0.56^{a}	16.3 ± 0.41^{ab}	16.2 ± 0.50^{ab}	19.4 ± 1.56^{bc}	20.3 ± 0.35^{c}
16:1n-7	3.71 ± 0.60^{a}	4.57 ± 0.23^{a}	7.29 ± 0.13^{b}	6.87 ± 0.09^{b}	3.84 ± 0.12^{a}	4.18 ± 0.21^{a}
17:0	2.61 ± 0.28	2.68 ± 0.05	2.47 ± 0.10	2.41 ± 0.14	2.51 ± 0.22	2.47 ± 0.04
18:0	11.2 ± 0.92^{b}	10.6 ± 0.63^{b}	7.88 ± 0.16^{a}	7.95 ± 0.29^{a}	10.1 ± 0.32^{ab}	10.2 ± 0.36^{ab}
18:1n-9	23.5 ± 1.37	22.9 ± 0.82	22.6 ± 0.38	25.7 ± 2.79	19.9 ± 1.12	19.6 ± 0.22
18:1n-11	11.9 ± 0.40^{c}	10.8 ± 0.63^{bc}	8.16 ± 0.14^{a}	8.25 ± 0.11^{a}	9.71 ± 0.60^{ab}	9.43 ± 0.14^{ab}
18:2n-6	4.80 ± 0.20	4.82 ± 0.22	4.45 ± 0.43	4.50 ± 0.39	4.02 ± 0.22	3.90 ± 0.22
18:3n-3	13.3 ± 2.55	13.9 ± 1.53	15.7 ± 0.09	12.2 ± 4.25	11.3 ± 0.94	11.6 ± 0.39
18:4n-3	3.60 ± 0.23^{c}	3.98 ± 0.33^{c}	1.86 ± 0.01^{a}	2.42 ± 0.09^{ab}	3.13 ± 0.10^{c}	3.30 ± 0.12^{c}
20:1	3.08 ± 0.22	1.96 ± 0.98	1.17 ± 1.17	Undetected	2.66 ± 0.19	1.76 ± 0.88
20:4n-6	0.58 ± 0.58^{ab}	Undetected	Undetected	0.64 ± 0.64^{ab}	2.26 ± 0.12^{b}	2.07 ± 0.08^{b}
20:5n-3	2.78 ± 0.13	2.69 ± 0.05	1.69 ± 0.86	2.53 ± 0.23	2.62 ± 0.39	2.89 ± 0.10
22:5n-3	Undetected	Undetected	2.11 ± 0.61	1.45 ± 0.73	1.44 ± 1.44	0.81 ± 0.81
22:6n-3	1.32 ± 1.32	Undetected	2.36 ± 0.16	2.60 ± 0.24	2.84 ± 0.27	1.78 ± 0.89
∑SFA	30.2 ± 1.56^{ab}	29.3 ± 0.53^{ab}	27.4 ± 1.29^{a}	27.6 ± 0.30^{a}	32.1 ± 1.20^{ab}	33.8 ± 0.52^{b}
∑MUFA	44.8 ± 1.79	42.9 ± 1.06	42.1 ± 1.08	43.7 ± 2.93	39.4 ± 1.63	38.4 ± 0.67
∑n-3 PUFA	19.6 ± 2.67	21.9 ± 0.61	23.8 ± 0.84	21.2 ± 3.68	20.9 ± 0.44	20.4 ± 0.20
∑n-6 PUFA	5.38 ± 0.76	5.76 ± 0.79	6.70 ± 1.46	7.46 ± 0.81	6.95 ± 0.65	7.45 ± 0.72
Total lipid mg/g	291 ± 14.5	294 ± 23.8	329 ± 16.2	267 ± 11.9	260 ± 27.0	299 ± 17.0

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 7
Total lipid and fatty acid composition (% of total fatty acids) (mean ± SEM, $N = 3$) of 13 dph red porgy larvae at the beginning of the experiment and on 25 dph after feeding *Artemia* enriched with two different enrichment products, Algamac 3000 and DC DHA Selco, and an unenriched (control) group.

Fatty acids	13 dph	25 dph		
		Control	DC DHA Selco	Algamac 3000
16:0	17.4 ± 0.34^{b}	14.2 ± 0.35^{a}	14.0 ± 0.39^{a}	14.3 ± 0.16^{a}
16:1n-7	6.24 ± 0.30^{b}	3.54 ± 0.15^{a}	4.37 ± 0.21^{a}	3.53 ± 0.25^{a}
17:0	3.12 ± 0.29	2.65 ± 0.10	2.47 ± 0.22	2.74 ± 0.33
18:0	9.83 ± 0.19^{a}	10.8 ± 0.44^{b}	9.30 ± 0.21^{a}	10.3 ± 0.09^{b}
18:1n-9	Undetected	20.0 ± 2.69	19.4 ± 1.49	16.2 ± 1.90
18:1n-11	6.39 ± 0.09	9.12 ± 0.55^{b}	7.71 ± 0.16^{a}	8.23 ± 0.12^{ab}
18:2n-6	5.42 ± 0.39	5.53 ± 0.11	6.90 ± 0.03	5.09 ± 0.18
18:3n-3	12.9 ± 0.51	6.04 ± 3.20	5.24 ± 1.19	7.64 ± 1.66
18:4n-3	Undetected	3.02 ± 0.14	2.35 ± 0.21	2.81 ± 0.30
20:0	Undetected	2.98 ± 0.13^{b}	Undetected	0.50 ± 0.50^{a}
20:1	3.52 ± 0.11	3.90 ± 0.12	2.18 ± 0.78	1.63 ± 0.94
20:3	Undetected	Undetected	0.77 ± 0.71	2.50 ± 0.83
20:4n-6	4.45 ± 0.21^{a}	4.63 ± 0.07^{a}	4.10 ± 0.67^{a}	5.42 ± 0.15^{b}
20:5n-3	8.01 ± 0.07^{bc}	6.92 ± 0.27^{a}	8.02 ± 0.14^{c}	7.24 ± 0.13^{ab}
22:5n-3	5.40 ± 0.62	Undetected	Undetected	Undetected
22:5n-6	Undetected	Undetected	Undetected	3.07 ± 0.27
22:6n-3	11.8 ± 0.03^{d}	3.55 ± 0.22^{a}	10.3 ± 0.23^{c}	7.09 ± 0.20^{b}
∑SFA	30.3 ± 0.65^{bc}	30.6 ± 0.65^{c}	25.7 ± 0.48^{a}	27.8 ± 0.45^{b}
∑MUFA	21.6 ± 1.38^{a}	37.3 ± 3.48^{b}	33.6 ± 0.82^{b}	30.4 ± 2.34^{b}
∑n-3 PUFA	38.1 ± 1.03^{b}	21.9 ± 1.96^{a}	28.9 ± 1.31^{a}	25.7 ± 1.50^{a}
∑n-6 PUFA	9.86 ± 0.59^{a}	10.2 ± 0.16^{a}	11.0 ± 0.19^{a}	13.6 ± 0.59^{b}
Total lipid mg/g	306 ± 57.8^{b}	172 ± 2.21^{a}	165 ± 7.67^{a}	173 ± 12.3^{a}

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.

(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-6 PUFAs 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 8
Total lipid and fatty acid composition (% of total fatty acids) (mean ± SEM, $N = 3$) of 33 dph red porgy larvae fed *Artemia* enriched with two different enrichment products, Algamac 3000 and DC DHA Selco and an unenriched (control) group.

Fatty acids	33 dph		
	Control	DC DHA Selco	Algamac 3000
16:0	13.3 ± 0.12	13.5 ± 0.63	14.3 ± 0.04
16:1n-7	5.59 ± 1.19b	3.90 ± 0.38^{ab}	2.83 ± 0.13^{a}
17:0	1.88 ± 1.88	2.03 ± 0.07	2.09 ± 0.05
18:0	10.1 ± 0.44	8.64 ± 0.76	10.3 ± 0.10
18:1n-9	12.6 ± 0.01^{a}	19.8 ± 1.54^{b}	15.8 ± 1.14^{ab}
18:1n-11	7.90 ± 0.74	7.07 ± 0.36	7.75 ± 0.14
18:2n-6	7.03 ± 0.64^{b}	6.18 ± 0.60^{ab}	4.73 ± 0.13^{a}
18:3n-3	9.49 ± 0.24	8.60 ± 0.93	11.1 ± 0.77
18:4n-3	1.85 ± 1.85	2.12 ± 0.04	2.33 ± 0.03
20:0	1.84 ± 1.84	0.99 ± 0.58	2.23 ± 0.05
20:1	5.40 ± 1.19^{b}	1.44 ± 0.83^{a}	Undetected
20:3	Undetected	1.49 ± 0.86	3.08 ± 0.03
20:4n-6	6.66 ± 0.65^{b}	3.42 ± 0.38^{a}	4.52 ± 0.11^{a}
20:5n-3	7.63 ± 0.55^{b}	6.23 ± 0.21^{a}	5.79 ± 0.11^{a}
22:5n-3	1.75 ± 1.75	1.82 ± 0.61	1.36 ± 0.46
22:5n-6	Undetected	0.85 ± 0.85^{a}	3.28 ± 0.08^{b}
22:6n-3	6.40 ± 1.46^{a}	10.6 ± 0.79^{b}	8.59 ± 0.23^{ab}
∑SFA	27.2 ± 3.51	25.1 ± 1.94	28.8 ± 0.15
∑MUFA	31.5 ± 3.12	33.5 ± 2.54	26.8 ± 1.12
∑n-3 PUFA	27.1 ± 1.36	29.4 ± 0.73	29.2 ± 0.81
∑n-6 PUFA	13.7 ± 1.29^{b}	10.5 ± 0.67^{a}	12.5 ± 0.27^{ab}
Total lipid mg/g	152 ± 23.8	126 ± 13.9	167 ± 25.4

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 9
Survival (%), total length (mm) and wet body weight (mg) of red porgy larvae fed UNCW MBD and commercial microdiets for 16 days. Values are means ± SEM ($N = 3$). No significant differences among diet treatments were observed for these parameters.

Parameter	Feeding period (day)	Microdiet		
		Gemma	UNCW	Otohime
Total length (mm)	16	13.7 ± 0.5	13.5 ± 0.4	14.4 ± 0.4
Body weight (mg)	16	47.06 ± 5.06	46.11 ± 4.17	53.65 ± 5.28
Survival (%)	7	55 ± 5	60 ± 2	56 ± 8
	12	49 ± 6	52 ± 6	46 ± 6
	16	39 ± 9	45 ± 8	43 ± 11

Table 10
Fatty acid composition of red porgy larvae fed different microdiets (% of total fatty acids). Values are means ± SEM ($N = 3$). Means in a row not sharing a letter in common are significantly ($P < 0.05$) different.

Fatty acids	Gemma	UNCW	Otohime
16:1n-7	2.18 ± 0.03	2.63 ± 0.19	2.86 ± 0.25
16:0	12.7 ± 0.29	12.9 ± 0.06	13.6 ± 0.27
18:4n-3	1.26 ± 0.11	1.23 ± 0.17	1.30 ± 0.08
18:2n-6	8.01 ± 0.49^{b}	6.60 ± 0.33^{ab}	5.79 ± 0.40^{a}
18:3n-3	7.33 ± 0.97	7.35 ± 0.31	7.83 ± 0.78
18:1n-9	14.3 ± 0.91	13.8 ± 0.83	11.3 ± 0.60
18:1n-11	4.38 ± 0.04	4.49 ± 0.17	4.43 ± 0.18
18:0	7.47 ± 0.08	7.54 ± 0.20	6.98 ± 0.08
20:4n-6	2.43 ± 0.08^{b}	2.38 ± 0.10^{ab}	2.07 ± 0.06^{a}
20:5n-3	7.00 ± 0.09^{a}	7.43 ± 0.21^{ab}	7.98 ± 0.27^{b}
20:0	0.75 ± 0.02	0.78 ± 0.05	0.70 ± 0.04
20:1	2.19 ± 0.06	2.09 ± 0.08	2.46 ± 0.12
22:6n-3	11.7 ± 0.37	10.7 ± 1.02	12.0 ± 0.43
22:5n-3	1.70 ± 0.12	1.89 ± 0.13	1.84 ± 0.04
SUM of n-3 PUFA	31.4 ± 0.76	31.0 ± 1.33	33.0 ± 0.80
SUM of n-6 PUFA	10.4 ± 0.50^{b}	8.98 ± 0.23^{ab}	7.85 ± 0.44^{a}

that concentrations of PUFAs were modestly improved by bioencapsulation. Lower than expected amounts of EPA in all rotifer treatments could in part be a result of the rotifers converting some EPA (20:5n-3) to DPA (22:5n-3), which ranged from 1.10 to 1.90% among treatments (Table 3). ARA levels were highest in rotifers enriched with Algamac + ARA and comparatively low in rotifers fed Algamac and DHA Protein Selco, both of which were devoid of ARA. The small amount of ARA found in rotifers enriched with Algamac and DHA Protein Selco (both ARA deficient) may also reflect the fact that rotifers were raised on RotiGrow which contained 3.2% ARA. A number of reports describe a direct relationship between PUFA levels in rotifers and in their emulsion diets (Watanabe et al., 1983; Rainuzzo et al., 1989; Koven et al., 2001b; Rezek et al., 2010; Carrier et al., 2011). The surprisingly low enrichment levels observed in the present study are presumably related to factors affecting uptake of emulsion media by rotifers, such as excessive pre-enrichment feeding levels, insufficient enrichment time, temperature, dissolved oxygen, pH, or other conditions that may have influenced bioencapsulation of nutrients.

Consistent with very minor differences in lipid and fatty acid composition of rotifers enriched with the different products (Rotifer Diet, DC DHA Protein Selco, Algamac 3000, Algamac 3000 + ARA) in this study, there were no significant main or interactive effects of rotifer enrichment media and feeding frequency on larval growth, or survival on any sampling day for the duration of the study. Final (18 dph) survival among all treatments was 22.8%, considered moderate for culture of other larval marine finfish such as southern flounder and black sea bass raised under similar conditions (Daniels and Watanabe, 2003; Watanabe, 2011). The lack of obvious detrimental effects of rotifers fed Rotifer Diet (which contained undetectable levels of DHA) indicate that red porgy larvae could be successfully reared on rotifers with relatively low levels of PUFA and with DHA: EPA ratios (0.27–0.75:1) lower than the recommended minimum of 2:1 DHA:EPA for marine finfish larvae (Tocher and Sargent 1984; Sargent et al., 1999). This further suggested that larvae fed 0% DHA may have derived a significant amount of DHA from maternal yolk reserves, since marine fish eggs normally contain high concentration of PUFAs, especially DHA (Tocher et al., 1985; Sargent 1995; Bransden et al., 2007). Alternatively, DHA may have been synthesized in the larvae from precursor fatty acids 18:3n-3, 20:5n-3 or 22:5n-3. Conversion of 18:3n-3 and 20:5n-3 to 22:6n-3 (DHA), while limited in most marine carnivorous fish species, has been reported in turbot *Scophthalmus maximus* (Linares and Henderson 1991; Mourente et al., 1991), gilthead seabream *Sparus aurata* (Mourente et al., 1993), Senegalese sole *Solea senegalensis* (Morais et al., 2012) and the southern flounder (Watanabe et al., 2014). In this study, the microalga *N. oculata* (31.4% EPA, 0% DHA and 3.94% ARA, Reed Mariculture Inc., Campbell, CA) was added to the larval rearing tanks as a background and may have also supplied 20:5n-3 and 20:4n-6 to the larvae either directly, or indirectly via rotifers. Since rotifers enriched with Rotifer Diet (*N. oculata* and *T. chuii*), showed no detectable DHA (Table 5), it is unlikely that the fish larvae acquired DHA (directly or indirectly via rotifers) through *N. oculata* used in background microalgae.

Although growth and survival patterns and stress resistance levels were similar among all rotifer enrichment treatments, distinctive cumulative survival patterns among treatments were observed under hyposalinity challenge and food deprivation, with consistently greater mean SAI values for larvae fed rotifers enriched with Algamac + ARA followed by Algamac, DHA Protein Selco, and then Rotifer Diet and for larvae fed 2×/day than for larvae fed 1×/day (Fig. 1). Due to high inter-replicate variability on each sampling date, statistically significant differences in SAI among enrichment treatments were not observed. However, SAI was positively correlated with DHA levels in rotifers enriched with these

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 Algamac + 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 hypersalinity stress and hypo-osmoregulatory ability (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 Algamac were higher than those found in unenriched control *Artemia*, reflecting relative DHA levels found in these enrichment products (Algamac = 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 (Sorgeloos 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 PUFAs, concentration of ARA was higher in *Artemia* enriched with Algamac 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* (Coutteau 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 Algamac 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 Algamac 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 quinqueradiata* (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 Algamac 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., 2009). 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 Otohime 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 Rodriganez et al., 2011).

In this study, the low survival (39–45%) of red porgy larvae 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 (Salhi 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 larvae 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 larvae 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 juveniles.

Acknowledgements

This study was supported by grants from the North Carolina Fishery Resource Grant Program (08-AM-02), the United States Department of Agriculture, National Institute of Food and Agriculture (USDA-NIFA Grant No. 2008-38854-18896) and Marine Biotechnology in North Carolina (MARBIONC). We thank Troy C. Rezek, Patrick M. Carroll, Walker D. Wright-Moore, and Tyler F. Gibson for technical assistance.

References

Ako, H., Tamaru, C.S., Bass, P., Lee, C.-S., 1994. Enhancing the resistance to physical stress in larvae of *Mugil cephalus* by the feeding of enriched *Artemia nauplii*. Aquaculture 122, 81–90.

Alam, M.S., Watanabe, W.O., Rezek, T.C., Myers, A.R., Carroll, P.M., Daniels, H.V., 2013. Growth performance and survival of larval southern flounder *Paralichthys lethostigma* fed commercial microdiets or formulated zein bound microdiets containing different protein sources. Aquac. Res., 1–13, http://dx.doi.org/10.1111/are.12347.

Andrade, C.A.P., Nascimento, F., Conceicao, L.E.C., Linares, F., Lacuisse, M., Dinis, M.T., 2012. Red porgy, *Pagrus pagrus*, larvae performance and nutritional condition in response to different weaning regimes. J. World Aquac. Soc. 43, 321–334.

AOAC (Association of Official Analytical Chemists), (2000). In: Horwitz, W., (Ed.) Official Methods of Analysis of AOAC, International 17th edition vols. 1 and 2. AOAC International, Arlington, Virginia.

Baskerville-Bridges, B., Kling, L.J., 2000. Early weaning of Atlantic cod (*Gadus morhua*) larvae onto a microparticulate diet. Aquaculture 189, 109–117.

Bengtson, D.A., 1993. A comprehensive program for the evaluation of artificial diets. J. World Aquac. Soc. 24, 285–293.

Bentley, C.D., Carroll, P.M., Riedel, A.M., Watanabe, W.O., 2008. Intensive rotifer production in a pilot-scale continuous culture recirculating system using condensed microalgae. J. World Aquac. Soc. 39, 625–635.

Bransden, M.N.P., Battaglene, S.C., Goldsmid, R.M., Dunstan, G.A., Nichols, P.D., 2007. Broodstock condition, egg morphology and lipid content and composition during the spawning season of captive striped trumpeter, *Latris lineata*. Aquaculture 268, 2–12.

Buke, E., Akpinar, A., Bulent, A., Dereli, H., 2005. Spawning performance and larval rearing of red porgy (*Pagrus pagrus* L., 1758) under culture conditions. E.U. J. Fish. Aquat. Sci. 22, 303–309.

Cahu, C., Zambonino-Infante, J., 2001. Substitution of live food by formulated diets in marine fish larvae. Aquaculture 200, 161–180.

Carrier III, J.K., Watanabe, W.O., Harel, M., Rezek, T.C., Seaton, P.J., Shafer, T.H., 2011. 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*. Aquaculture 319, 111–121.

Cavalin, F.G., Weirich, C.R., 2009. Larval performance of aquacultured Florida pompano (*Trachinotus carolinus*) fed rotifers (*Brachionus plicatilis*) enriched with selected commercial diets. Aquaculture 292, 67–73.

Cho, S.H., Lim, Y.S., Lee, J.H., Lee, J.K., Park, S., Lee, S.M., 2003. Effects of feeding rate and feeding frequency on survival, growth, and body composition of ayu post-larvae *Plecoglossus altivelis*. J. World Aquac. Soc. 34, 85–91.

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

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

Daniels, H.V., Watanabe, W.O., 2003. A Practical Hatchery Manual: Production of Southern Flounder Fingerlings. Sea Grant Publication, North Carolina, pp. 40.

Evjemo, J.O., Coutteau, P., Olsen, Y., Sorgeloos, P., 1997. The stability of docosahexaenoic acid in two *Artemia* species following enrichment and subsequent starvation. Aquaculture 155, 135–148.

Faulk, C.K., Holt, G.J., 2005. Advances in rearing cobia *Rachycentron canadum* larvae in recirculating aquaculture systems: live prey enrichment and greenwater culture. Aquaculture 249, 231–243.

Faulk, C.K., Holt, G.J., Davis, D.A., 2005. Evaluation of fatty acid enrichment of live food for yellowtail snapper *Ocyurus chrysurus* larvae. J. World Aquac. Soc. 36, 271–281.

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

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

Garcia, A.S., Parrish, C.C., Brown, J.A., 2008. A comparison among differently enriched rotifers (*Brachionus plicatilis*) and their effect of Atlantic cod (*Gadus morhua*) larvae early growth, survival and lipid composition. Aquac. Nutr. 14, 14–30.

Henne, J.P., Watanabe, W.O., 2003. Effects of light intensity and salinity on growth, survival and osmoregulatory ability of southern flounder larvae *Paralichthys lethostigma*. J. World Aquac. Soc. 34, 450–465.
Hernandez-Cruz, C.M., Salhi, M., Bessonart, M., Izquierdo, M.S., Gonzalez, M.M., Fernandez-Palacios, H., 1999. Rearing techniques for red porgy (*Pagrus pagrus*) during larval development. Aquaculture 179, 489–497.
Izquierdo, M., Fernandez-Palacios, H., 2001. Nutritional requirements of marine fish larvae and broodstock. CIHEAM Options Mediterr., 243–264.
Izquierdo, M.S., Watanabe, T., Takeuchi, T., Arakawa, T., Kitajima, C., 1989. Requirement of larval red seabream, *Pagrus major*, for essential fatty acids. Nippon Suisan Gakkaishi 55, 859–886.
Kanazawa, A., Koshio, S., Teshima, S., 1989. Growth and survival of larval red sea bream *Pagrus major* and Japanese flounder *Paralichthys olivaceus* fed microbound diets. J. World Aquac. Soc. 20, 31–37.
Kim, S.-K., Takeuchi, T., Akimoto, A., Furuita, H., Yamamoto, T., Yokoyama, M., Murata, Y., 2005. Effect of taurine supplemented practical diet on growth performance and taurine contents in whole body and tissues of juvenile Japanese flounder, *Paralichthys olivaceus*. Fish. Sci. 71, 627–632.
Kolios, P., Kiritsis, S., Katribusas, N., 1997. Larval-rearing and growout of the red porgy (*Pagrus pagrus*) in the Riopesca hatchery (Greece). Hydrobiologia 358, 321–325.
Kolkovski, S., 2000. Amino acids as food attractants. 21st congress of the European society for comparative physiology and biochemistry, Liege, Belgium. Comp. Biochem. Physiol. 126, 80.
Kolkovski, S., 2001. Digestive enzymes in fish larvae and juveniles—implications and applications to formulated diets. Aquaculture 200, 181–201.
Kolkovski, S., Tandler, A., 2000. The use of squid protein hydrolysate as a protein source in microdiet for gilthead seabream *Sparus aurata* larvae. Aquac. Nutr. 6, 11–15.
Kolkovski, S., Arieli, A., Tandler, A., 1995. Visual and olfactory stimuli as determining factors in the stimulation of microdiet ingestion in gilthead seabream *Sparus aurata* larvae. In: Lavens, P., Jaspers, E., Roelants, I. (Eds.), Larvi'95—Fish and Shellfish Symposium. European Aquaculture Society Spec. Pub. 24, Gent, Belgium, pp. 289–292.
Kolkovski, S., Arieli, A., Tandler, A., 1997. Visual and chemical cues stimulate microdiet ingestion in sea bream larvae. Aquac. Int. 5, 527–536.
Kolkovski, S., Lazzo, J.P., Leclercq, D., Izquierdo, M., 2009. Larval nutrition and diets: new developments. In: Burnell, G., Allan, G. (Eds.), New Technologies in Aquaculture: Improving Production Efficiency, Quality and Environmental Management. Woodhead Publishing Limited, Cambridge, UK, pp. 315–369, 78.
Koven, W., Kolkovski, S., Hadas, E., Gamsiz, K., Tandler, A., 2001a. Advances in the development of MD's for gilthead seabream, *Sparus aurata*: a review. Aquaculture 194, 107–121.
Koven, W., Barr, Y., Lutzky, S., Ben-Atia, I., Weiss, R., Harel, M., Behrens, P., Tandler, A., 2001b. The effects of dietary arachidonic acid (20:4n-6) on growth, survival and resistance to handling stress in gilthead seabream (*Sparus aurata*). Aquaculture 193, 107–122.
Kramer, C.Y., 1956. Extension of multiple range tests to group means with unequal number of replications. Biometrics 12, 307–310.
Kvale, A., Yufera, M., Nygard, E., Aursland, K., Harboe, T., Hamre, K., 2006. Leaching properties of three different microparticulate diets and preference of the diets in cod (*Gadus morhua* L.) larvae. Aquaculture 28, 402–415.
Langdon, C., Clack, B., Onal, U., 2007. Complex microparticles for delivery of low molecular weight, water soluble nutrients and pharmaceuticals to marine fish larvae. Aquaculture 268, 143–148.
Lian, P.L., Lee, C.M., Bengtson, D., 2008. Development of a squid-hydrolysate-based larval diet and its feeding performance on summer flounder, *Paralichthys dentatus*, larvae. J. World Aquac. Soc. 39, 196–204.
Linares, F., Henderson, R.J., 1991. Incorporation of ^{14}C-labelled polyunsaturated fatty acids by juvenile turbot, *Scophthalmus maximus* L. *in vivo*. J. Fish Biol. 38, 335–347.
Lopez-Alvarado, J., Kanazawa, A., 1994. Effect of dietary arginine levels on growth of red sea bream larvae fed diets supplemented with crystalline arginine. Fish. Sci. 60, 435–439.
Mangino, A., Watanabe, W.O., 2006. Combined effects of turbulence and salinity on growth, survival and whole-body osmolality of larval southern flounder. J. World Aquac. Soc. 37, 407–418.
Matsuo, Y., Kasahara, Y., Hagiwara, A., Sakakura, Y., Arakawa, T., 2006. Evaluation of larval quality of viviparous scorpionfish *Sebastiscus marmoratus*. Fish. Sci. 72, 948–954.
McEvoy, L., Navarro, J.C., Bell, J.G., Sargent, J.R., 1995. Autoxidation of oil emulsions during the *Artemia* enrichement process. Aquaculture 134, 101–112.
Mihelakakis, A., Yoshimatsu, T., Tsolkas, C., 2001a. Effects of feeding rate on growth, feed utilization and body composition of red porgy fingerlings: preliminary results. Aquac. Int. 9, 237–245.
Mihelakakis, A., Yoshimatsu, T., Tsolkas, C., 2001b. Spawning in captivity and early life history of cultured red porgy, *Pagrus pagrus*. Aquaculture 199, 333–352.
Morais, S., Castanheira, F., Martinez-Rubio, L., Conceicao, L.E.C., Tocher, D.R., 2012. Long chain polyunsaturated fatty acid synthesis in a marine vertebrate: ontogenetic and nutritional regulation of a fatty acyl desaturase with △4 activity. Biochim. Biophys. Acta 1821, 660–671.
Morris Jr., J.A., Rezek, T.C., McNeill, N.A., Watanabe, W.O., 2008. Aquaculture of the Atlantic red porgy. North Am. J. Aquac. 70, 184–191.
Mourente, G., Tocher, D.R., Sargent, J.R., 1991. Specific accumulation of docosahexanoic acid (22:6n-3) in brain lipids during development of juvenile turbot *Scophthalmus maximus* L. Lipids 26, 871–877.
Mourente, G., Rodriguez, A., Tocher, D.R., Sargent, J.R., 1993. Effects of dietary docosahexaenoic acid (22:6n-3) on lipid and fatty acid compositions and growth in gilthead sea bream (*Sparus aurata* L.) larvae during the first feeding. Aquaculture 112, 79–98.
Moustakas, C.Th., Watanabe, W.O., Copeland, K.A., 2004. Combined effects of photoperiod and salinity on growth, survival, and osmoregulatory ability of southern flounder *Paralichthys lethostigma*. Aquaculture 229, 159–179.
Nankervis, L., Southgate, P.C., 2006. An integrated assessment of gross marine protein sources used in formulated microbound diets for barramundi (*Lates calcarifer*) larvae. Aquaculture 257, 453–464.
Ostrowski, A.D., Watanabe, W.O., Montgomery, F.A., Rezek, T.C., Shafer, T.H., Morris Jr., J., 2011. Effects of salinity and temperature on the growth, survival, whole body osmolality, and expression of Na^+/K^+ ATPase mRNA in red porgy (*Pagrus pagrus*) larvae. Aquaculture 314, 193–201.
Papandroulakis, N., Kentouri, M., Divanch, P., 2004. Biological performance of red porgy (*Pagrus pagrus*) larvae under intensive rearing conditions with the use of an automated feeding system. Aquac. Int. 12, 191–203.
Parker, R.O., Dixon, R.L., 1998. Changes in a North Carolina reef fish community after 15 years of intense fishing—global warming implications. Trans. Am. Fish. Soc. 127, 908–920.
Rabe, J., Brown, J.A., 2000. A pulse feeding strategy for rearing larval fish: an experiment with yellowtail flounder. Aquaculture 191, 289–302.
Rainuzzo, J.R., Olsen, Y., Rosenlund, G., 1989. The effect of enrichment diets on the fatty acids composition of the rotifer *Brachionus placatilis*. Aquaculture 79, 157–161.
Rezek, T.C., Watanabe, W.O., Harel, M., Seaton, P.J., 2010. Effects of dietary docosahexaenoic acid (22:6n-3) and arachidonic acid (20:4n-6) on the growth, survival, stress resistance and fatty acid composition in black seabass *Centropristis striata* (Linnaeus 1758) larvae. Aquac. Res. 41, 1302–1314.
Roo, E.J., Izquierdo, M.S., Caballero, M.J., Hernández-Cruz, C.M., Fernández, A., Fernández-Palacios, H., 1999. Development of red porgy *Pagrus pagrus* visual system in relation with changes in the digestive tract and larval feeding habits. Aquaculture 179, 499–512.
Roo, E.J., Hernandez-Cruz, C.M., Socorro, J.A., Fernandez-Palacios, H., Montero, D., Izquierdo, M.S., 2009. Effect of DHA content in rotifers on the occurrence of skeletal deformities in red porgy *Pagrus pagrus* (Linnaeus, 1758). Aquaculture 287, 84–93.
Roo, E.J., Hernández-Cruz, C.M., Socorro, J.A., Fernández-Palacios, H., Izquierdo, M.S., 2010. Advances in rearing techniques of *Pagrus pagrus*, (Linnaeus, 1758): comparison between intensive and semi-intensive larval rearing systems. Aquac. Res. 41, 433–449.
Russo, D.J., 2013. Effects of feeding frequency of live prey on larval growth, survival, resistance to hyposaline stress, Na^+/K^+ ATPase activity and fatty acid profiles in black sea bass *Centropristis striata*. M.S. Thesis. University of North Carolina Wilmington, Center for Marine Science, Wilmington, NC, USA.
SAFMC (South Atlantic Fishery Management Council) 2006. Stock assessment of red porgy off the southeastern United States. SouthEast Data, Assessment, and Review (SEDAR). Report of Assessment Workshop. Beaufort, North Carolina. April 4–5, 2006. http://www.sefsc.noaa.gov/sedar/download/rp-aw-25May06.pdf?id=DOCUMENT (accessed 07.09.06.).
SAFMC 2010. Fishing regulations for south Atlantic federal waters for species managed by the South Atlantic Fisheries Management Council. http://www.safmc.net/http://www.safmc.net/Council.
Saenz de Rodriganez, M.A., Gander, B., Alaiz, M., Moyano, F.J., 2011. Physico-chemical characterization and in vitro digestibility of commercial feeds used in weaning of marine fish. Aquac. Nutr. 17, 429–440.
Salhi, M., Bessonart, M., 2012. Growth, survival and fatty acid composition of *Rhamdia quelen* (Quoy and Gaimard, 1824) larvae fed on artificial diet alone or in combination with *Artemia nauplii*. Aquac. Res. 44, 41–49.
Sargent, J.R., 1995. Orgins and functions of egg lipids: nutritional implications. In: Bromage, N.R., Roberts, R.J. (Eds.), Broodstock Management and Egg and Larval Quality. Blackwell Science, Oxford, pp. 353–372.
Sargent, J.R., Bell, G., McEvoy, L., Tocher, D., Estevez, A., 1999. Recent developments in the essential fatty acid nutrition of fish. Aquaculture 177, 191–199.
Sorgeloos, P., Dhert, P., Candreva, P., 2001. Use of brine shrimp *Artemia* spp., in marine fish larviculture. Aquaculture 200, 147–159.
Takaoka, O., Takii, K., Nakamura, M., Kumai, H., Takeda, M., 1995. Identification of feeding stimulants for tiger puffer. Fish. Sci. 61, 833–836.
Teshima, S., Kanazawa, A., Sakamoto, M., 1982. Microparticulate diets for the larval of aquatic animals. Mini Rev. and data file of fisheries research. Kagoshima Univ. 2, 67–86.
Teshima, S., Ishikawa, M., Koshio, S., 2000. Nutritional assessment and feed intake of microparticulate diets in crustaceans and fish. Aquac. Res. 31, 691–702.
Teshima, S., Koshio, S., Ishikawa, M., Alam, M.S., Hernandez, H.L.H., 2004. Effects of protein and lipid sources on the growth and survival of red sea bream *Pagrus major* and Japanese flounder *Paralichthys olivaceus* receiving micro-bound diets during larval and early juvenile stage. Aquac. Nutr. 10, 279–287.
Tocher, D.R., Sargent, J.R., 1984. Analysis of lipids and fatty acids in ripe roes of some Northwest European marine fish. Lipids 7, 492–499.
Tocher, D.R., Fraser, A.J., Sargent, J.R., 1985. Fatty acid composition of phospholipids and neutral lipids during embryonic and early larval development in Atlantic herring *Clupea harengus*. Lipids 20, 69–74.
Vaughan, D.S., Prager, M.H., 2002. Severe decline in abundance of the red porgy (*Pagrus pagrus*) population off the southeastern United States. Fish. Bull. 100, 351–375.

Wang, Y., Hu, M., Wang, W., Cao, L., 2009. Effects on growth and survival of loach (*Misgurnus anguillicaudatus*) larvae when co-fed on live and microparticle diets. Aquac. Res. 40, 385–394.
Watanabe, T., Kitajima, C., Fujita, S., 1983. Nutritional values of live organisms used in Japan for mass propagation of fish: a review. Aquaculture 34, 115–143.
Watanabe, T., Izquierdo, M.S., Takeuchi, T., Satoh, S.Y., Kitajima, C., 1989. Comparison between eicosapentaenoic and docosahexaenoic acids in terms of essential fatty acid efficacy in larval red seabream. Bull. Jpn Soc. Sci. Fish. 55, 1635–1640.
Watanabe, W.O., 2011. Species profile: Black Sea Bass. SRAC Publication No. 7207. August 2011. Southern Regional Aquaculture Center. Texas A & M University. SRAC Publication No. 7207.
Watanabe, T., Kiron, V., 1994. Prospects in larval fish dietetics. Aquaculture 124, 223–251.
Watanabe, W.O., Rezek, T.C., Harel, M., Seaton, P.J., 2014. The effects of dietary arachidonic acid (20:4n-6) on growth, survival and hypersaline stress resistance in larval southern flounder *Paralichthys lethostigma* (Jordan and Gilbert 1884). In: O'Keefe, J.M. (Ed.), "Arachidonic Acid: Sources, Biosynthesis and Health Effects". Nova Science Publishers Inc., pp. 1–27 (Chapter 1) ISBN: 978-1-63117-619-7.
Woolley, L.D., Partridge, G.J., 2015. The effect of different rotifer feeding regimes on the growth and survival of yellowtail kingfish *Seriola lalandi* (Valenciennes, 1833) larvae. Aquac. Res., http://dx.doi.org/10.1111/are.12723.
Yufera, M., Pascual, E., Fernandez-Diaz, C., 1999. A highly efficient microencapsulated food for rearing early larvae of marine fish. Aquaculture 177, 249–256.