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Advances in rearing cobia *Rachycentron canadum* larvae in recirculating aquaculture systems: Live prey enrichment and greenwater culture

Cynthia K. Faulk*, G. Joan Holt

University of Texas at Austin Marine Science Institute, Fisheries and Mariculture Laboratory, 750 Channel View Drive, Port Aransas, TX 78373, United States

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Abstract

Cobia *Rachycentron canadum* is a relatively hardy species which exhibits high rates of growth during the larval and juvenile periods. Currently, this species is considered to be a good candidate for commercial production in recirculating aquaculture systems. However, little information is available regarding the nutritional requirements of cobia larvae in such systems, and this information is required to advance commercial technologies for the successful production of cobia fingerlings. Experiments were conducted to examine the effects of enriching rotifers and *Artemia* with live algae or commercial preparations on the growth and survival of cobia *R. canadum* larvae and to evaluate the benefits of adding live algae to the systems. Prey items were enriched with live *Isochrysis galbana*, live *Nannochloris oculata*, Algamac 2000, Algamac 2000 supplemented with 10% or 20% Aquagrow arachidonic acid, or Algamac 3050. In addition, larvae fed prey enriched with Algamac 2000 were reared in the presence of live *I. galbana* (~40,000 cells ml^{-1}) or *N. oculata* (~80,000 cells ml^{-1}). Significant differences in the fatty acid composition of the rotifers and *Artemia* were found among treatments. Generally, prey enriched with the commercial preparations contained higher levels of highly unsaturated fatty acids than those enriched with live algae. Furthermore, a positive correlation was found between dietary docosahexaenoic acid (DHA) and the amount of DHA measured in the whole body tissues of 16-day-old larvae. Larval growth (measured as standard length) and survival of 16-day-old larvae were significantly higher ($P<0.05$) when larvae were fed prey enriched with the commercial preparations (14.7–15.2 mm; 12.0–15.6%) compared to *N. oculata* (11.8 mm; 4.4%). However, when larvae were reared on *N. oculata* enriched rotifers and subsequently fed Algamac 2000 enriched *Artemia* there were no significant differences in growth or survival compared to larvae which were fed both rotifers and *Artemia* enriched with Algamac 2000. This suggests that the enrichment of rotifers may be less important than the enrichment of *Artemia*. No significant differences in growth or survival were found when larvae were fed prey enriched with live *I. galbana* (13.5 mm; 8.2%) or commercial preparations (12.4–12.6 mm; 12.9%). However, the presence of live algae (*I. galbana* or *N. oculata*) in the rearing tanks significantly improved larval survival to 23.3% and 24.7%, respectively. The results of this study suggest that

* Corresponding author. Tel.: +1 361 749 6808; fax: +1 361 749 6749.
E-mail address: cfaulk@utmsi.utexas.edu (C.K. Faulk).

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enriching rotifers and *Artemia* with live *I. galbana* or commercial preparations such as Algamac 2000 and 3050 in conjunction with greenwater culture systems improves the growth and survival of cobia larvae in recirculating aquaculture systems.
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Keywords: Cobia larvae; Prey enrichment; Greenwater culture

1. Introduction

Cobia are highly fecund, multiple batch spawners (Brown-Peterson et al., 2001) distributed worldwide, with the exception of the eastern Pacific Ocean, in tropical, subtropical, and temperate waters (Briggs, 1960). There has been considerable interest in developing reliable methods for spawning and rearing cobia in captivity because they are hardy, fast growing fish and are therefore a viable species for aquaculture (Franks et al., 1999). Cobia have been cultured in Taiwan since the early 1990s and represent an important part of the commercial industry (Liao et al., 2001). In the United States, captive broodstock have been induced to spawn with the use of hormonal injection (Franks et al., 2001) and the natural spawning of cobia in recirculating aquaculture systems has been carried out since 2001 (Arnold et al., 2002). Several studies have also reported successful larval rearing of cobia in semi-static and recirculating aquaculture systems from both wild caught (Hassler and Rainville, 1975) and captive spawned eggs (Faulk and Holt, 2003) with the use of rotifers, *Artemia*, and/or wild zooplankton. However, little information is available regarding the nutritional requirements of cobia larvae in recirculating aquaculture systems, and such information is essential to maximize larval growth and survival and further the successful commercial production of this species.

One important aspect of larval nutrition is providing adequate levels of highly unsaturated fatty acids (HUFAs) including arachidonic acid (ARA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3), and docosahexaenoic acid (DHA, 22:6n-3) (Sargent et al., 1999a). HUFAs play an important role in maintaining cell membrane structure and function, stress tolerance, and proper development and functioning of neural and visual systems (Kanazawa, 1997; Rainuzzo et al., 1997; Sargent et al., 1997). Several studies have shown that marine fishes are unable to convert shorter chain fatty acids such as linolenic acid (18:3n-3) and linoleic acid (18:2n-6) to longer chain HUFAs due to low activity of the necessary enzymes, thus making it necessary to provide these fatty acids through the diet (Mourente and Tocher, 1993; Ghioni et al., 1999). Recently, researchers have suggested that the biochemical composition of eggs and yolksac larvae reflect the basic nutritional requirements of first feeding larvae (Rainuzzo et al., 1997; Sargent et al., 1999b). Faulk and Holt (2003) examined the fatty acid composition of cobia *Rachycentron canadum* eggs and yolksac larvae and reported high levels of HUFAs with DHA, EPA, and ARA accounting for approximately 80% of the polyunsaturated fatty acids. This suggests that cobia larvae may require high levels of these fatty acids in their diets.

A common practice for rearing marine fish larvae in captivity is to feed rotifers for several days or weeks gradually switching them over to *Artemia* nauplii and finally weaning them onto a dry feed. As rotifers and *Artemia* are naturally deficient in HUFAs it is necessary to enrich these live feeds with essential fatty acids prior to offering them to the larvae (Sargent et al., 1997). Enrichments frequently employed to enhance the fatty acid composition of prey items include live microalgae, microalgae paste and lipid emulsions that include various marine fish and/or PUFA oils. Furthermore, microalgae are often added to the rearing tanks along with the live prey and have been shown to enhance larval growth and survival (Naas et al., 1992; Papandroulakis et al., 2002). The purpose of this study was to determine if enriching rotifers and *Artemia* with high levels of HUFAs would improve growth and survival of cobia larvae. In addition, the benefits of adding live algae to the larval rearing tanks were evaluated.

2. Materials and methods

Cobia larvae were shipped overnight from the Aquaculture Center of the Florida Keys, Inc. (Marathon, Florida, USA) to the Fisheries and Mariculture Laboratory of the University of Texas Marine Science Institute in Port Aransas, Texas and arrived 3 and 2 days after hatching for trials 1 and 2, respectively. Spawning salinity and temperature were 36.5 ppt and 26–27 °C. Salinity in the shipping containers was slowly decreased to 34.5 ppt at which point larvae were carefully moved into 150 l conical rearing tanks with the use of a 250 ml beaker (500 larvae $tank^{-1}$). The rearing tanks were equipped with internal biological filters to maintain water quality as described by Craig et al. (1990). The number of larvae surviving the transfer from the shipping containers to each tank was estimated by placing 10 larvae in each of five 1-l beakers and counting the number surviving 24 h later. The standard lengths of 20 larvae were measured to the nearest 0.1 mm using a Wild Heerbrugg stereomicroscope, Summa Sketch III digitizing tablet (GTCO CalComp, Inc., Columbia, Maryland, USA) and Sigma Scan software (Jandel Corporation, San Rafael, California, USA). Prior to measurement, larvae were anesthetized with 0.1% tricaine methanesulfonate. The mean standard length of larvae stocked into the rearing tanks was 4.6 ± 0.1 mm in trial 1 and 4.2 ± 0.1 mm in trial 2. Two replicates of approximately 200 larvae each were sampled for subsequent lipid analysis as described below for live prey.

Salinity and dissolved oxygen of the survival beakers and the rearing tanks were 34.5 ± 0.1 ppt and 6.1 ± 0.1 mg l^{-1}, respectively. Mean water temperature was 26.4 ± 0.2 °C during trial 1 and 26.0 ± 0.2 °C during trial 2. Photoperiod was set at 14-h light/10-h dark. Ammonia-nitrogen (Solorzano, 1969), nitrite-nitrogen (Strickland and Parsons, 1972), and pH were measured once a week and averaged $<0.1 \pm 0.1$ mg l^{-1}, $<0.1 \pm <0.1$ mg l^{-1}, and 7.9 ± 0.1, respectively, during the first 7 days of rearing and 0.3 ± 0.1 mg l^{-1}, $0.1 \pm <0.1$ mg l^{-1}, and 7.9 ± 0.1, respectively, thereafter. During trial 2, no effect of the live algae addition on water quality parameters was observed. Although the effects of ammonia and nitrite concentrations on the growth and survival of cobia larvae are unknown, the levels measured in this study are within acceptable ranges reported for other species (Holt and Arnold, 1983).

2.1. Rotifer and Artemia enrichments

Rotifers *Brachionus plicatilis* used in this study were obtained from a continuous culture raised on a combination of live *Nannochloris oculata* and concentrated algae paste (*Nannochloropsis oculata*; Reed Mariculture, Cambell, California, USA). Prey enrichment times were chosen to raise HUFA levels in rotifers and *Artemia* as suggested by the manufacturer of the commercial products and based upon preliminary studies conducted in our laboratory. Rotifers were harvested the day before feeding, placed in multiple 10 l plastic containers (300 rotifers ml^{-1}), and enriched twice over a 24-h period (0 and 15 h). *Artemia* cysts were incubated for 24 h at which time nauplii were separated from empty cysts, placed in 10 l plastic containers (50–100 nauplii ml^{-1}), and subsequently enriched three times over a 32-h period (0, 9, and 24 h). Prior to re-enrichment, rotifers and *Artemia* were collected on a 55 or 150 μm sieve, respectively, rinsed with clean seawater, and returned to the 10 l enrichment containers. This step was included in the process in order to remove any unused enrichment material remaining in the containers from the previous enrichment period. Commercial products were added to the enrichment containers at doses of 0.3 g per 1×10^6 rotifers and 0.2 g per 100,000 *Artemia* nauplii. Live algae enrichments were performed in concentrations of approximately 3.0×10^6 cells ml^{-1} for *Isochrysis galbana* and 2.0×10^7 cells ml^{-1} for *N. oculata*. Rotifers and *Artemia* were maintained at 30.0 ppt and 26.5 ± 0.2 °C throughout the study.

Enriched rotifers and *Artemia* were sampled on two separate occasions during the course of each feeding trial for lipid analysis and dry weight determinations. Samples were collected 19 and 28 h into the enrichment process for rotifers and *Artemia*, respectively. After collection, samples were placed in a 50 ml cylinder (PVC) with a 48 μm nitex mesh bottom, rinsed three times with 20 ml of distilled water, transferred to pre-weighed glass vials, and frozen at −80 °C. Once frozen, samples were lyophilized, reweighed for determination of dry

weights, and the vials were flushed with nitrogen and held at -80 °C until lipid extraction could be performed.

2.2. Feeding study

For both feeding trials, three replicate rearing tanks were set for each treatment. Prey concentrations were chosen based upon the results of preliminary studies which indicated that the following feeding schedule produced good growth and survival for larval densities used in this study. Equal amounts of rotifers (approximately 2.0 ml^{-1}) were added to the rearing tanks three times a day on days 3–7 after hatching. Newly hatched *Artemia* nauplii (<0.5 ml^{-1}) were added once in the morning on days 7–8. Finally, enriched *Artemia* nauplii (approximately 0.5 ml^{-1}) were added three times a day on day 8 through the end of the experiment.

Trial 1 included the following enrichments: Algamac 2000 (A2000), Algamac 2000 supplemented with 10% Aquagrow arachidonic acid (ARA10), Algamac 2000 supplemented with 20% Aquagrow arachidonic acid (ARA20), and live *N. oculata*. Algamac 2000 and Aquagrow arachidonic acid were obtained from Aquafauna Bio-Marine (Hawthorne, California, USA) and Advanced BioNutrition (Columbia, Maryland, USA), respectively. In addition, a treatment with mass cultured rotifers grown on *N. oculata* followed by Algamac 2000 enriched *Artemia* (Art) was included in the study to evaluate the need for enrichment of rotifers. In the second feeding trial, larvae were offered enriched prey items with or without the addition of live algae to the rearing tanks in 5 treatment combinations. In the first 3 treatments, both rotifers and *Artemia* were enriched with either A2000, Algamac 3050 (A3050; Aquafauna Bio-Marine, Hawthorne, California, USA), or live *I. galbana*. The 2 remaining treatments were greenwater culture treatments with larvae receiving rotifers and *Artemia* enriched with Algamac 2000 and either *I. galbana* (Algae-I) or *N. oculata* (Algae-N) added to the rearing tanks at concentrations of $40{,}000 \pm 5{,}000$ and $80{,}000 \pm 10{,}000$ cells ml^{-1}, respectively.

On days 7 and 16 after hatching, larvae ($n = 20$) were randomly collected from each rearing tank for measurement of standard length and fatty acid analysis. Day 7 was chosen for sampling because it marks the end of the rotifer feeding period and beginning of the *Artemia* feeding period. In our laboratory, successful weaning of cobia larvae onto commercial feeds has been achieved by co-feeding the larvae *Artemia* and dry feed for several days beginning on day 16. Therefore, day 16 was chosen for sampling larvae while they were feeding exclusively on *Artemia*. Larvae were collected in the morning prior to feeding. Standard length measurements and sample processing for fatty acid analysis were performed as described above. Survival was estimated by taking into consideration the number of larvae placed in each tank, 24 h larval survival, the number of larvae removed on day 7 for sampling, and the number of larvae remaining in each tank at the end of the experiment (day 16).

2.3. Fatty acid analysis

Total lipids were cold extracted from lyophilized samples with chloroform/methanol (2:1, v/ v) as described by Folch et al. (1957) and 0.01% (w/ v) butylated hydroxytoluene was added as an antioxidant. Crude lipid extracts from the live prey enrichments were measured gravimetrically following evaporation of the solvent under nitrogen. Total lipids were saponified in 0.5 M KOH and fatty acid methyl esters (FAMEs) were prepared by transesterification with 14% boron trifluoride in methanol following the procedure of Morrison and Smith (1964).

FAMEs were analyzed on a Hewlett-Packard 5890A gas chromatograph equipped with a flame ionization detector and a Supelcowax 10 fused silica capillary column (30 m long, 0.53 mm internal diameter, 1.0 μm thickness; Supelco, Inc., Bellefonte, Pennsylvania, USA). Helium was used as a carrier gas at the rate of 4 ml min^{-1} and a split ratio of 10:1. Injector and detector temperatures were 250 and 260 °C, respectively. Oven temperature was held at 160 °C for 5 min and rose to 220 °C at a rate of 3 °C min^{-1} where it was held for 30 min. FAME peaks were recorded with the use of a Tigre II analog/digital interface and Chrom Perfect Spirit software (Justice Laboratory Software, Palo Alto, California, USA). Individual peaks were identified by comparison to chromatograms of known standards (Supelco, Inc., Bellefonte, Pennsylvania, USA).

Table 1
Total lipid content (mg lipid/g dry weight) and selected fatty acid composition (% total fatty acids) of rotifers fed different enrichments

Fatty acid	A2000	ARA10	ARA20	A3050	*N. oculata*	*I. galbana*
18:2n-6	$1.8\pm<0.1^{a}$	1.7 ± 0.1^{a}	$1.7\pm<0.1^{a}$	$0.8\pm<0.1^{a}$	10.5 ± 0.5^{b}	12.9 ± 1.2^{b}
18:3n-3	$1.0\pm<0.1^{a}$	$1.0\pm<0.1^{a}$	$0.9\pm<0.1^{a}$	$0.4\pm<0.1^{a}$	7.2 ± 1.1^{b}	6.3 ± 0.1^{b}
20:4n-6	$2.4\pm<0.1^{a,b}$	$3.0\pm0.1^{a,c}$	3.9 ± 0.1^{d}	$3.4\pm0.1^{c,d}$	$1.9\pm0.3^{b,e}$	1.4 ± 0.1^{e}
20:5n-3	4.3 ± 0.1	4.6 ± 0.2	4.0 ± 0.3	4.2 ± 0.1	5.8 ± 1.2	3.7 ± 0.3
22:5n-3	$2.6\pm<0.1$	2.8 ± 0.1	2.2 ± 0.2	2.4 ± 0.1	4.0 ± 1.0	2.5 ± 0.3
22:5n-6	6.9 ± 0.2^{a}	7.2 ± 0.2^{a}	6.6 ± 0.4^{a}	12.3 ± 0.3^{b}	0.9 ± 0.3^{c}	0.4 ± 0.2^{c}
22:6n-3	20.4 ± 0.1^{a}	22.4 ± 1.1^{a}	18.4 ± 1.4^{a}	31.5 ± 1.1^{b}	$0.1\pm<0.1^{c}$	3.4 ± 0.3^{d}
∑HUFA	$32.3\pm0.3^{a,b}$	$35.1\pm1.4^{a,b}$	30.4 ± 2.0^{a}	44.0 ± 1.4^{b}	16.9 ± 2.4^{c}	16.9 ± 1.5^{c}
n-3 HUFA	$29.2\pm0.3^{a,b}$	31.3 ± 1.5^{a}	25.9 ± 1.9^{c}	39.6 ± 1.4^{a}	$12.9\pm2.4^{b,c}$	$13.4\pm1.4^{b,c}$
n-6 HUFA	3.1 ± 0.1^{a}	$3.8\pm0.1^{b,c}$	4.5 ± 0.1^{d}	$4.4\pm<0.1^{c,d}$	$4.0\pm<0.1^{b,d}$	$3.5\pm0.1^{a,b}$
DHA/EPA	4.7 ± 0.1^{a}	$4.9\pm<0.1^{a}$	4.5 ± 0.1^{a}	7.6 ± 0.2^{b}	$0.1\pm<0.1^{c}$	$0.9\pm<0.1^{d}$
EPA/ARA	$1.8\pm<0.1^{a}$	$1.5\pm0.1^{a,b}$	1.0 ± 0.1^{b}	$1.2\pm<0.1^{b}$	3.0 ± 0.2^{c}	2.6 ± 0.1^{c}
Total lipid	207.9 ± 7.5	200.5 ± 11.0	198.3 ± 5.6	210.9 ± 15.4	187.2 ± 5.1	210.5 ± 17.8

Values (mean ± S.E.M.) followed by different superscript letters within a row are significantly different ($P<0.05$).
A2000 = Algamac 2000; ARA10 = 90% Algamac 2000 + 10% Aquagrow arachidonic acid; ARA20 = 80% Algamac 2000 + 20% Aquagrow arachidonic acid; A3050 = Algamac 3050.
HUFA = highly unsaturated fatty acids defined as fatty acids with at least 20 carbon atoms and two or more double bonds.

2.4. Statistical analysis

A one-way ANOVA followed by a Tukey test for multiple comparisons of means was used for each feeding trial to test for significant differences ($P<0.05$) among treatments in larval growth, survival, prey lipid content, and the fatty acid composition of prey and cobia larvae. Data was transformed as necessary to meet the normality assumptions of ANOVA. Linear regression was performed to evaluate the incorporation of select dietary fatty acids from enriched *Artemia* nauplii into larval tissues. Data are expressed as mean ± S.E.M. Statistical analyses were performed using SYSTAT 10.0 (SPSS Inc., 2000, Chicago, IL, USA).

3. Results

No statistical differences in individual fatty acids were found for similar prey items enriched with Algamac 2000 alone irrespective of feeding trial.

Table 2
Total lipid content (mg lipid/g dry weight) and selected fatty acid composition (% total fatty acids) of *Artemia* fed different enrichments

Fatty acid	A2000	ARA10	ARA20	A3050	*N. oculata*	*I. galbana*
18:2n-6	3.4 ± 0.2^{a}	3.6 ± 0.4^{a}	3.5 ± 0.4^{a}	$2.6\pm<0.1^{a}$	$5.6\pm<0.1^{b}$	7.7 ± 1.2^{c}
18:3n-3	$12.9\pm3.3^{a,b}$	$16.3\pm1.6^{a,b}$	$15.8\pm1.7^{a,b}$	12.4 ± 0.2^{b}	23.0 ± 0.6^{c}	$18.8\pm1.2^{a,c}$
20:4n-6	2.4 ± 0.1^{a}	2.5 ± 0.2^{a}	3.7 ± 0.1^{b}	3.8 ± 0.1^{b}	1.3 ± 0.1^{c}	1.2 ± 0.1^{c}
20:5n-3	5.5 ± 0.3^{a}	$5.9\pm0.4^{a,b}$	$5.9\pm0.4^{a,b}$	$7.2\pm01^{a,b}$	7.5 ± 0.3^{b}	3.5 ± 0.3^{c}
22:5n-3	$0.2\pm<0.1^{a,b}$	$0.1\pm0.1^{a,b}$	$0.1\pm0.1^{a,b}$	0.6 ± 0.2^{a}	$0.1\pm<0.1^{b}$	$0.3\pm0.2^{a,b}$
22:5n-6	4.1 ± 1.3^{a}	2.7 ± 0.4^{a}	2.5 ± 0.6^{a}	9.5 ± 0.8^{b}	$<0.1\pm<0.1^{c}$	0.7 ± 0.3^{d}
22:6n-3	13.4 ± 0.8^{a}	12.8 ± 0.2^{a}	12.8 ± 0.5^{a}	21.3 ± 0.6^{b}	$0.1\pm<0.1^{c}$	2.8 ± 0.8^{d}
∑HUFA	23.1 ± 0.8^{a}	22.9 ± 0.7^{a}	24.7 ± 0.7^{a}	35.0 ± 1.0^{b}	11.2 ± 0.4^{c}	9.6 ± 0.7^{c}
n-3 HUFA	20.3 ± 0.8^{a}	20.0 ± 0.5^{a}	20.3 ± 0.7^{a}	30.6 ± 0.8^{b}	9.3 ± 0.3^{c}	7.9 ± 0.9^{c}
n-6 HUFA	2.8 ± 0.2^{a}	2.9 ± 0.2^{a}	4.4 ± 0.3^{b}	4.4 ± 0.2^{b}	1.8 ± 0.1^{c}	1.6 ± 0.2^{c}
DHA/EPA	2.5 ± 0.2^{a}	$2.2\pm0.1^{a,c}$	$2.2\pm<0.1^{a,c}$	3.0 ± 0.1^{a}	$0.1\pm<0.1^{b}$	0.8 ± 0.5^{c}
EPA/ARA	$2.3\pm<0.1^{a,b}$	2.4 ± 0.1^{a}	1.6 ± 0.1^{c}	$1.9\pm0.1^{b,c}$	5.7 ± 0.4^{d}	$3.0\pm<0.1^{e}$
Total lipid	191.9 ± 0.1	204.6 ± 4.7	190.2 ± 14.2	194.6 ± 9.1	203.5 ± 12.5	193.9 ± 7.7

Values (mean ± S.E.M.) followed by different superscript letters within a row are significantly different ($P<0.05$).
A2000 = Algamac 2000; ARA10 = 90% Algamac 2000 + 10% Aquagrow arachidonic acid; ARA20 = 80% Algamac 2000 + 20% Aquagrow arachidonic acid; A3050 = Algamac 3050.
HUFA = highly unsaturated fatty acids defined as fatty acids with at least 20 carbon atoms and two or more double bonds.

Table 3
Selected fatty acid composition (% total fatty acids) of cobia larvae prior to exogenous feeding (day 3) and following 4 days of feeding on rotifers enriched with live algae or commercial preparations (day 7) during trial 1

Fatty acid	Day 3	Day 7				
		A2000	ARA10	ARA20	*N. oculata*	Art
18:2n-6	1.9 ± 0.1	1.7 ± 0.2[a]	1.7 ± 0.2[a]	1.9 ± 0.1[a]	4.6 ± 0.1[b]	4.7 ± 0.3[b]
18:3n-3	0.5 ± <0.1	0.5 ± 0.1[a]	0.6 ± 0.2[a]	0.7 ± 0.2[a]	2.3 ± 0.2[b]	2.5 ± 0.3[b]
20:4n-6	3.3 ± 0.2	4.5 ± 0.3	4.3 ± 0.3	4.5 ± 0.3	4.0 ± 0.6	3.8 ± 0.5
20:5n-3	5.8 ± 0.3	3.7 ± 1.4	2.4 ± 0.1	2.6 ± 0.3	3.5 ± 0.1	3.9 ± 0.1
22:5n-3	1.6 ± 0.2	2.5 ± 0.2[a]	2.4 ± 0.2[a]	2.6 ± 0.4[a,b]	3.5 ± 0.3[a,b]	3.8 ± 0.1[b]
22:5n-6	0.9 ± 0.1	4.2 ± 0.3[a]	4.3 ± 0.3[a]	3.8 ± 0.4[a]	0.7 ± 0.1[b]	0.7 ± 0.1[b]
22:6n-3	27.2 ± 2.4	18.9 ± 1.8	20.4 ± 1.6	19.8 ± 2.5	16.7 ± 1.8	15.9 ± 2.7
∑HUFA	38.7 ± 3.1	31.7 ± 1.7	30.8 ± 2.2	31.7 ± 3.2	30.3 ± 2.8	30.7 ± 3.6
n-3 HUFA	35.0 ± 2.9	26.0 ± 2.0	25.9 ± 1.9	26.1 ± 3.0	25.3 ± 2.1	26.0 ± 2.9
n-6 HUFA	3.7 ± 0.2	5.7 ± 0.4	4.9 ± 0.3	5.6 ± 0.4	5.1 ± 0.7	4.7 ± 0.8
DHA/EPA	4.7 ± 0.2	6.4 ± 1.9	8.7 ± 0.9	7.7 ± 0.9	4.8 ± 0.4	4.1 ± 0.7
EPA/ARA	1.7 ± <0.1	0.9 ± 0.4	0.6 ± 0.1	0.6 ± 0.1	0.9 ± 0.1	1.1 ± 0.2

Values (mean ± S.E.M.) for day 7 larvae followed by different superscript letters within a row are significantly different ($P < 0.05$). A2000 = Algamac 2000; ARA10 = 90% Algamac 2000 + 10% Aquagrow arachidonic acid; ARA20 = 80% Algamac 2000 + 20% Aquagrow arachidonic acid; Art = rotifers enriched with *N. oculata* and *Artemia* enriched with Algamac 2000.
HUFA = highly unsaturated fatty acids defined as fatty acids with at least 20 carbon atoms and two or more double bonds.

Therefore, data collected from the Algamac 2000 enrichment in trials 1 and 2 were pooled for statistical comparisons among enrichments. Overall, the use of live algae and commercial enrichments resulted in significantly different fatty acid profiles for both rotifers (Table 1) and *Artemia* (Table 2) while no significant differences in the total lipid content were detected.

The fatty acid composition of rotifers and *Artemia* enriched with the commercial preparations A2000, ARA10, ARA20, and A3050 closely resembled one another except for a significant increase in the level of DHA in the A3050 treatment for both rotifers and *Artemia*. As a result, the levels of total HUFAs, n-3 HUFAs, and the ratio of DHA/EPA were also higher in the A3050 treatment. The substitution of 20% Aqua-

Table 4
Selected fatty acid composition (% total fatty acids) of cobia larvae prior to exogenous feeding (day 2) and following 4 days of feeding on rotifers enriched with live algae or commercial preparations (day 7) with or without the addition of live algae to the rearing tanks during trial 2

Fatty acid	Day 2	Day 7				
		A2000	A3050	Algae-I	Algae-N	*I. galbana*
18:2n-6	3.2 ± 0.2	1.4 ± 0.1[a,b]	0.9 ± 0.2[a]	2.7 ± 0.1[b,c]	2.8 ± 0.3[c]	3.3 ± 0.4[c]
18:3n-3	0.6 ± <0.1	0.7 ± 0.1[a]	0.6 ± 0.1[a]	0.6 ± <0.1[a]	0.6 ± 0.1[a]	1.8 ± 0.2[b]
20:4n-6	4.1 ± 0.1	3.9 ± 0.5	3.3 ± 0.1	3.1 ± 0.2	4.3 ± 0.3	3.9 ± 0.1
20:5n-3	8.1 ± 0.1	3.2 ± 0.2	3.5 ± <0.1	2.7 ± 0.6	4.0 ± 0.2	4.4 ± 0.3
22:5n-3	1.9 ± 0.1	2.6 ± 0.1	2.4 ± 0.3	3.4 ± 0.4	2.8 ± 0.1	2.5 ± 0.2
22:5n-6	0.9 ± <0.1	3.7 ± 0.5	2.0 ± 0.5	1.3 ± 0.1	2.2 ± 0.2	0.7 ± 0.5
22:6n-3	26.3 ± 0.4	19.4 ± 1.3	18.3 ± 1.5	18.6 ± 1.2	19.2 ± 1.0	16.8 ± 1.0
∑HUFA	41.2 ± 0.5	34.6 ± 0.4	35.2 ± 0.4	35.4 ± 2.3	32.3 ± 0.9	32.4 ± 0.6
n-3 HUFA	36.8 ± 0.4	24.8 ± 0.9	25.0 ± 0.8	25.8 ± 1.8	25.6 ± 1.0	23.9 ± 0.9
n-6 HUFA	4.4 ± 0.1	4.8 ± 1.2	5.2 ± 1.2	4.6 ± 0.5	4.7 ± 0.1	5.0 ± 0.8
DHA/EPA	3.3 ± <0.1	6.0 ± 0.5	5.2 ± 0.5	7.4 ± 2.0	4.8 ± <0.1	3.9 ± 0.2
EPA/ARA	2.0 ± <0.1	0.8 ± 0.1	1.1 ± <0.1	0.8 ± 0.1	0.9 ± 0.1	1.1 ± 0.1

Values (mean ± S.E.M.) for day 7 larvae followed by different superscript letters within a row are significantly different ($P < 0.05$).A2000 = Algamac 2000; A3050 = Algamac 3050; Algae-I = greenwater culture using *I. galbana* and prey enriched with Algamac 2000; Algae-N = greenwater culture using *N. oculata* and prey enriched with Algamac 2000.
HUFA = highly unsaturated fatty acids defined as fatty acids with at least 20 carbon atoms and two or more double bonds.

Table 5
Selected fatty acid composition (% total fatty acids) of cobia larvae (day 16) following 7 days of feeding on *Artemia* enriched with live algae or commercial preparations during trial 1

Fatty acid	A2000	ARA10	ARA20	*N. oculata*	Art
18:2n-6	2.8 ± 0.1^{a}	2.8 ± 0.1^{a}	$2.8 \pm <0.1^{a}$	4.8 ± 0.1^{b}	2.8 ± 0.1^{a}
18:3n-3	8.5 ± 0.2^{a}	9.0 ± 0.6^{a}	8.8 ± 0.4^{a}	11.9 ± 0.5^{b}	8.6 ± 0.3^{a}
20:4n-6	5.0 ± 0.1	4.4 ± 0.1	4.5 ± 0.1	4.5 ± 0.4	4.7 ± 0.2
20:5n-3	4.2 ± 0.1	4.8 ± 0.9	4.5 ± 0.2	6.4 ± 0.9	4.1 ± 0.1
22:5n-3	$1.1 \pm <0.1^{a}$	$0.9 \pm <0.1^{b}$	1.1 ± 0.1^{a}	$1.2 \pm <0.1^{a}$	$1.1 \pm <0.1^{a}$
22:5n-6	4.1 ± 0.1^{a}	4.2 ± 0.1^{a}	4.0 ± 0.1^{a}	0.5 ± 0.2^{b}	4.1 ± 0.2^{a}
22:6n-3	13.3 ± 0.2^{a}	13.4 ± 0.2^{a}	13.3 ± 0.3^{a}	1.9 ± 0.4^{b}	12.9 ± 0.1^{a}
∑HUFA	25.5 ± 0.1^{a}	25.7 ± 0.8^{a}	25.2 ± 0.3^{a}	16.9 ± 0.9^{b}	24.8 ± 0.5^{a}
n-3 HUFA	19.9 ± 0.1^{a}	20.8 ± 0.9^{a}	20.4 ± 0.5^{a}	11.7 ± 1.2^{b}	19.6 ± 0.3^{a}
n-6 HUFA	5.6 ± 0.2	4.9 ± 0.1	4.9 ± 0.2	5.2 ± 0.4	5.3 ± 0.4
DHA/EPA	$3.2 \pm <0.1^{a}$	2.9 ± 0.5^{a}	3.0 ± 0.2^{a}	$0.3 \pm <0.1^{b}$	3.2 ± 0.1^{a}
EPA/ARA	$0.8 \pm <0.1$	1.1 ± 0.2	1.0 ± 0.1	1.5 ± 0.3	0.9 ± 0.1

Values (mean ± S.E.M.) followed by different superscript letters within a row are significantly different ($P<0.05$).
A2000 = Algamac 2000; ARA10 = 90% Algamac 2000 + 10% Aquagrow arachidonic acid; ARA20 = 80% Algamac 2000 + 20% Aquagrow arachidonic acid; Art = rotifers enriched with *N. oculata* and *Artemia* enriched with Algamac 2000.
HUFA = highly unsaturated fatty acids defined as fatty acids with at least 20 carbon atoms and two or more double bonds.

grow ARA to the Algamac 2000 enrichment medium significantly increased the amount of ARA in both prey items while a 10% substitution did not. The increase in ARA observed in the ARA20 treatment resulted in a subsequent increase in n-6 HUFAs and a decrease in the EPA/ARA ratio. The amount of n-3 and total HUFAs measured in *Artemia* nauplii enriched with the commercial preparations was lower than that measured in similarly enriched rotifers apparently due to a substantial drop in DHA content. Consequently, DHA/EPA ratios were depressed in *Artemia* nauplii compared to rotifers in treatments utilizing the commercial preparations. Compared to the commercial enrichments, prey items enriched with live algae had significantly lower levels of DHA, docosapentaenoic acid (DPA, 22:5n-6), n-3 HUFAs, and DHA/EPA ratios, especially the *N. oculata* treatment which contained only trace amounts of DHA and DPA.

In larvae, fewer significant differences in fatty acids were found among treatments at 7 days after

Table 6
Selected fatty acid composition (% total fatty acids) of cobia larvae following 7 days of feeding on *Artemia* enriched with live algae or commercial preparations (day 16) with or without the addition of live algae to the rearing tanks during trial 2

Fatty acid	A2000	A3050	Algae-I	Algae-N	*I. galbana*
18:2n-6	3.3 ± 0.1^{a}	$2.8 \pm <0.1^{a}$	4.4 ± 0.3^{b}	$3.4 \pm <0.1^{a}$	8.4 ± 0.5^{c}
18:3n-3	10.4 ± 0.6	6.6 ± 2.8	10.6 ± 0.3	9.3 ± 0.3	10.5 ± 1.1
20:4n-6	$4.7 \pm 0.4^{a,b}$	5.8 ± 0.2^{b}	$4.7 \pm 0.1^{a,b}$	4.4 ± 0.1^{a}	3.4 ± 0.6^{a}
20:5n-3	5.0 ± 0.4	5.4 ± 0.2	6.2 ± 1.0	4.3 ± 0.2	5.8 ± 0.8
22:5n-3	1.1 ± 0.1	1.3 ± 0.1	$1.2 \pm <0.1$	1.1 ± 0.1	1.1 ± 0.3
22:5n-6	4.3 ± 0.4^{a}	7.2 ± 0.1^{b}	3.9 ± 0.5^{a}	$4.8 \pm 0.1^{a,b}$	1.3 ± 0.3^{c}
22:6n-3	$12.3 \pm 1.3^{a,b}$	15.9 ± 0.4^{a}	10.9 ± 1.1^{b}	$13.5 \pm 0.8^{a,b}$	3.7 ± 0.3^{c}
∑HUFA	25.2 ± 0.5^{a}	30.4 ± 0.4^{b}	25.0 ± 0.9^{a}	25.2 ± 0.4^{a}	17.0 ± 1.5^{c}
n-3 HUFA	19.9 ± 0.8^{a}	24.0 ± 0.4^{b}	19.8 ± 1.0^{a}	20.3 ± 0.4^{a}	12.7 ± 0.7^{c}
n-6 HUFA	$5.3 \pm 0.5^{a,b}$	6.3 ± 0.1^{a}	$5.3 \pm 0.1^{a,b}$	$4.9 \pm 0.1^{a,b}$	4.3 ± 0.7^{b}
DHA/EPA	$2.5 \pm 0.4^{a,b}$	2.9 ± 0.1^{a}	$1.9 \pm 0.5^{a,b}$	3.2 ± 0.3^{a}	0.7 ± 0.1^{b}
EPA/ARA	$1.1 \pm 0.1^{a,b}$	$0.9 \pm <0.1^{a}$	$1.3 \pm 0.2^{a,b}$	1.0 ± 0.1^{a}	1.7 ± 0.1^{b}

Values (mean ± S.E.M.) followed by different superscript letters within a row are significantly different ($P<0.05$).
A2000 = Algamac 2000; A3050 = Algamac 3050; Algae-I = greenwater culture using *I. galbana* and prey enriched with Algamac 2000; Algae-N = greenwater culture using *N. oculata* and prey enriched with Algamac 2000.
HUFA = highly unsaturated fatty acids defined as fatty acids with at least 20 carbon atoms and two or more double bonds.

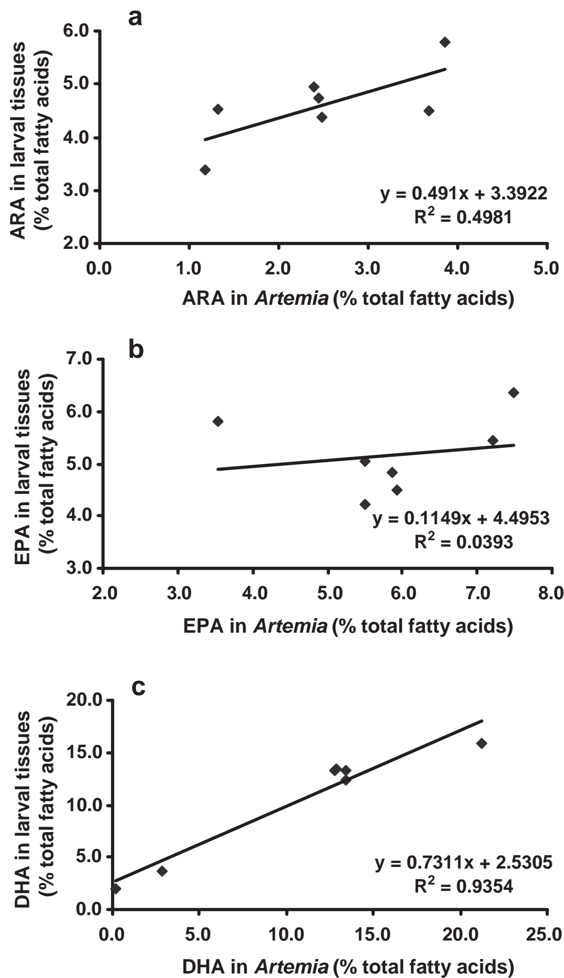

Fig. 1. Correlation between the percentage of ARA (a), EPA (b), and DHA (c) in *Artemia* nauplii enriched with live algae or commercial products and the percentage of corresponding fatty acids measured in the whole body tissues of 16-day-old cobia larvae.

hatching (Tables 3 and 4) than at 16 days after hatching (Tables 5 and 6). In fact, the fatty acid profiles of day 7 cobia larvae more closely resembled that of larvae prior to the onset of exogenous feeding than the profiles of the enriched rotifers that they fed on. For example, the amount of DHA measured in rotifers enriched with commercial preparations was significantly higher than those enriched with live algae, especially *N. oculata*, yet no significant decrease in DHA or the ratio of DHA/EPA was detected in the tissues of 7-day-old larvae in the *N. oculata* treatment. One exception to this trend was the DPA levels measured in the whole body tissues of day 7 larvae in trial 1 (Table 3) which were higher in larvae fed rotifers enriched with commercial preparations than in larvae fed rotifers enriched with *N. oculata*. By day 16 post hatch, the fatty acid composition of the larvae more closely resembled that of the live prey. Larvae feeding on *Artemia* enriched with commercial preparations contained higher levels of DHA, DPA, n-3 HUFAs, and total HUFAs in their tissues than those feeding on prey enriched with live algae. As a result, the ratio of DHA/EPA was also higher in fish from these treatments.

Fig. 1 shows a comparison between the DHA, EPA, and ARA content of enriched *Artemia* nauplii and that found in the larval tissue. As the amount of DHA increased in the live prey, a corresponding increase was evident in the larval tissue. However, a significant correlation between the dietary levels of EPA and ARA and matching tissue levels was not observed.

In trial 1, the mean standard length of 7-day-old cobia larvae fed rotifers enriched with *N. oculata* was significantly lower (5.7 mm; Table 7) than those fed rotifers enriched with the commercial preparations (6.1 mm). Similarly, at 16 days after hatching the size of larvae offered prey enriched with the commercial preparations (14.7–15.2 mm) was significantly greater than those offered prey fed *N. oculata* (11.8 mm). In addition, the standard length of larvae fed rotifers enriched with *N. oculata* but *Artemia* enriched with A2000 (14.0 mm; Art treatment) was significant-

Table 7
Standard length (days 7 and 16) and survival (day 16) of cobia larvae fed live prey enriched with live algae or commercial preparations during trial 1

Enrichment	Standard length (mm)		Survival (%)
	Day 7	Day 16	Day 16
A2000	$6.1 \pm <0.1^{a}$	15.2 ± 0.5^{a}	12.0 ± 1.1^{a}
ARA10	$6.1 \pm <0.1^{a}$	14.7 ± 0.4^{a}	13.0 ± 2.3^{a}
ARA20	6.1 ± 0.1^{a}	15.2 ± 0.2^{a}	15.6 ± 1.2^{a}
N. oculata	5.7 ± 0.1^{b}	11.8 ± 0.3^{b}	4.4 ± 1.8^{b}
Art	5.7 ± 0.1^{b}	14.0 ± 0.3^{a}	11.6 ± 1.0^{a}

Values (mean ± S.E.M.) followed by different superscript letters within a column are significantly different ($P<0.05$).
A2000 = Algamac 2000; ARA10 = 90% Algamac 2000 + 10% Aquagrow arachidonic acid; ARA20 = 80% Algamac 2000 + 20% Aquagrow arachidonic acid; Art = rotifers enriched with *N. oculata* and *Artemia* enriched with Algamac 2000.

Table 8
Standard length (days 7 and 16) and survival (day 16) of cobia larvae fed live prey enriched with varying levels of HUFA or reared under greenwater culture conditions during trial 2

Enrichment	Standard length (mm)		Survival (%)
	Day 7	Day 16	Day 16
A2000	5.5 ± <0.1	12.6 ± 0.7	12.9 ± 2.3[a]
A3050	5.5 ± 0.1	12.4 ± 0.1	12.9 ± 2.1[a]
Algae-I	5.7 ± <0.1	12.7 ± 0.5	23.3 ± 1.4[b]
Algae-N	5.5 ± 0.1	11.4 ± 0.7	24.7 ± 3.0[b]
I. galbana	5.7 ± 0.2	13.5 ± 1.5	8.2 ± 0.7[a]

Values (mean ± S.E.M.) followed by different superscript letters within a column are significantly different ($P < 0.05$).
A2000 = Algamac 2000; A3050 = Algamac 3050; Algae-I = greenwater culture using *I. galbana* and prey enriched with Algamac 2000; Algae-N = greenwater culture using *N. oculata* and prey enriched with Algamac 2000.

ly greater than those fed rotifers and *Artemia* enriched with *N. oculata* alone. The percent of larvae surviving from day 3 through the end of the experiment was significantly lower for larvae in the *N. oculata* treatment (4.4%) as compared to the remaining 4 treatments with values ranging from 11.6% to 15.6%. No significant difference in survival was found among the A2000, ARA10, ARA20, and Art treatments even though larvae in the Art treatment were initially fed rotifers enriched with *N. oculata*.

The mean standard length of larvae reared during trial 2 was not significantly different among treatments at 7 or 16 days after hatching and ranged from 5.5 to 5.7 mm at day 7 and 11.4 to 13.5 at day 16 (Table 8). Larval survival at the end of the experiment was significantly higher when the larvae were reared in a greenwater culture irrespective of the type of algae added to the rearing tanks (*N. oculata* or *I. galbana*). Percent survival averaged 23.3 and 24.7% in the Algae-I and Algae-N treatments, respectively. No significant differences were detected in larval survival among the A2000, A3050, and *I. galbana* treatments with values ranging from 8.2% to 12.9%.

4. Discussion

Successful rearing of marine fish larvae is partially dependent upon the proper presentation of lipids, proteins, carbohydrates, vitamins, and minerals via the diet (Watanabe and Kiron, 1994; Kanazawa, 2003). The aim of this study was to examine the effect of different live algae and commercial enrichments, readily available to producers, on larval growth and survival and not to define the precise dietary requirements of cobia larvae. Although it is likely that the nutritional composition of the live prey varied in factors other than fatty acid composition, valid comparisons can be made between the live algae enrichments and among the commercial preparations. Palmtag (2004) found no significant differences in the protein, carbohydrate, or total lipid content of rotifers and *Artemia* enriched with live algae and commercial preparations similar to those used in this study including live *I. galbana*, live *N. oculata*, and Algamac 2000. Aragao et al. (2004) fed rotifers and *Artemia* different types of enrichments and subsequently examined the composition of the free amino acid (FAA) and protein-bound amino acid (PAA) pools of the prey. The FAA pool of rotifers and *Artemia* more closely resembled that of the enrichment material than the PAA pool in which only minor differences were noted among enrichments. Weltzien et al. (1999) reported that the FAA pool of rotifers and *Artemia* represents only a small portion of the total amino acids (~5%). This suggests that the enrichments used in this study would result in relatively minor differences in the overall amino acid composition of prey especially when the compositions of the enrichments themselves are also taken into consideration. Brown et al. (1997) examined the nutritional properties of over 40 species of microalgae, including *I. galbana* and *N. oculata*, and reported that all species had similar amino acid compositions. Information provided by the manufacturer of the commercial preparations indicates that the vitamin and amino acid content of Algamac 2000 and Algamac 3050 are also similar.

Each of the different enrichments employed in this study resulted in significantly different patterns in the fatty acid composition of rotifers and *Artemia*. In comparison to the live algae enrichments, the commercial preparations resulted in higher levels of essential fatty acids known to enhance the growth and survival of fish larvae. Furthermore, the substitution of 20% Aquagrow arachidonic acid to Algamac 2000 significantly increased the level of this fatty acid in the live prey. Differences between enrichment types were more pronounced in the fatty acid profiles of rotifers than *Artemia*. These results are not surprising

as the difficulties associated with enriching *Artemia* nauplii with high HUFA levels, especially DHA, has been well documented. Navarro et al. (1999) enriched *Artemia* with radiolabelled DHA and found a marked retroconversion of this fatty acid to EPA after a 24-h enrichment period. Han et al. (2001) demonstrated that *Artemia* exhibit a lower incorporation efficiency of DHA than EPA and ARA. Although previous studies have suggested that there is also a difference between the incorporation efficiency of DHA and EPA during the enrichment of rotifers (Estevez et al., 1999), the variation does not appear to be as severe as that found during the enrichment of *Artemia* (Harel et al., 2002). The combination of decreased incorporation efficiency of DHA and its ready retroconversion to EPA help explain the differences among the fatty acid profiles of *Artemia* enriched with commercial preparations compared to rotifers.

The amount of DHA detected in the tissue of day 16 cobia larvae fed live prey enriched with *N. oculata* was 1.9% of the total fatty acids even though *Artemia* nauplii contained negligible levels of DHA. These results are consistent with the findings of previous studies conducted on marine fish larvae and may be the result of chain elongation/desaturation of shorter chain fatty acids or preferential retention of DHA in larval tissues. Estevez et al. (1999) found evidence of chain elongation of EPA to DHA in turbot *Scophthalmus maximus* larvae fed live prey containing relatively high levels of EPA and low levels of DHA. Blair et al. (2003) reported significant retention of DHA and ARA in the whole body tissues of haddock *Melanogrammus aeglefinus* larvae fed live feeds or formulated diets containing varying HUFA levels. In this study, a significant correlation between dietary levels of EPA and ARA and those measured in the larval tissues was not observed in day 16 cobia larvae. The variation in the amount of EPA and ARA among different *Artemia* enrichments was fairly minor and may not have been sufficient to observe a significant correlation between dietary input and larval tissues in these fatty acids. The incorporation of DHA from enriched *Artemia* into larval tissues was positively correlated with levels measured in the dietary lipids indicating preferential retention of this fatty acid. Similar results were reported for red drum *Sciaenops ocellatus* larvae (Brinkmeyer and Holt, 1998). However, the findings of this study do not rule out the possibility that cobia are capable of some chain elongation/desaturation of shorter chain fatty acids to longer chain HUFAs.

Numerous studies conducted on a variety of marine fishes including yellowtail flounder *Limanda ferruginea* (Copeman et al., 2002), Japanese flounder *Paralichthys olivaceus* (Furuita et al., 1999), and red drum (Craig et al., 1994) have shown that dietary increases of DHA enhance larval growth. As previously mentioned, it has been suggested that the biochemical composition of eggs and yolksac larvae may reflect the nutritional requirements of marine fish larvae (Rainuzzo et al., 1997; Sargent et al., 1999b). In this study, the DHA content of cobia larvae prior to exogenous feeding (days 2 and 3) was approximately 26.5% of the total fatty acids. The percent of DHA measured in prey enriched with live algae was considerably lower measuring 0.1% and 3.4% in rotifers and 0.1% and 2.8% in *Artemia* enriched with *N. oculata* and *I. galbana*, respectively. Even so, larval growth and survival was significantly greater in the *I. galbana* treatment compared to the *N. oculata* treatment. Prey enriched with the commercial preparations contained significantly higher levels of DHA which more closely resembled levels measured in the pre-feeding larvae. The most significant difference in the fatty acid profiles of prey in the ARA20 and A3050 treatments was the level of DHA which increased from 18.1% to 31.5% in rotifers and 12.8% to 21.3% in *Artemia*. However, concurrent increases in growth and survival were not observed. These results suggest that cobia larvae, in terms of growth and survival, have a requirement for the inclusion of DHA in their diet although it may be less than that suggested by the high levels observed in eggs and yolksac larvae.

Previous studies have shown that higher dietary levels of ARA enhance larval growth. For example, Bessonart et al. (1999) found that increasing the amount of ARA in the larval feed from 0.1% to 1.0% dry weight resulted in significantly higher growth rates for larval gilthead seabream *Sparus auratus*. In this study, the addition of an ARA supplement to the Algamac 2000 enrichment increased the level of ARA in live prey from 2.4% to 3.9% for rotifers and 2.4% to 3.7% in *Artemia* which is similar to the 3.5% ARA measured in day 2 and 3 cobia larvae (prior to exogenous feeding). However, no significant differ-

ences in the amount of ARA was measured in the whole body tissues of day 7 and 16 cobia larvae nor where any differences in growth and survival detected among the A2000, ARA10, and ARA20 treatments. Rotifers and *Artemia* enriched with Algamac 2000 or Algamac 2000+ARA also contained approximately 7.0% and 2.7% DPA, respectively. Koven et al. (2001) found evidence of the retroconversion of DPA into ARA in gilthead seabream larvae. If cobia also retro-convert DPA into ARA it may have masked any positive effect of the ARA supplementation on larval growth and survival and would help explain the similarity in ARA tissue levels among the A2000, ARA10, and ARA20 treatments.

Interestingly, when larvae reared on mass cultured rotifers fed *N. oculata* (low HUFAs) were subsequently fed Algamac 2000 enriched *Artemia* (Art treatment) there were no significant differences in growth or survival at day 16 compared to larvae which were fed Algamac 2000 enriched rotifers and *Artemia* (A2000 treatment). This suggests that, in terms of growth and survival, the enrichment of rotifers may be less important than the enrichment of *Artemia* perhaps due to the short period of time cobia larvae are fed rotifers. However, it is important to note previous studies such as those conducted on mahimahi *Coryphaena hippurus* (Kraul et al., 1993) and gilthead seabream larvae (Koven et al., 2003) which have shown that increasing the levels of dietary HUFAs increases the stress resistance of the larvae. Therefore, cobia larvae fed prey containing limited amounts of HUFAs, such as those enriched with *N. oculata* and *I. galbana*, may be less resistant to stressors commonly present in commercial production settings such as frequent handling and high rearing densities which were not factors in this study.

The presence of live algae (*I. galbana* or *N. oculata*) in cobia rearing tanks significantly improved larval survival at day 16 but did not have an obvious affect on growth. Increased growth and survival as a result of greenwater culture have been reported for a number of marine fish larvae including halibut *Hippoglossus hippoglossus* (Naas et al., 1992), sea bream (Papandroulakis et al., 2002), and turbot (Oie et al., 1997). The beneficial effects associated with the addition of algae have been attributed to a variety of factors including the direct consumption of the algae by larvae (Reitan et al., 1998), maintaining the nutritional quality of live prey (Reitan et al., 1997), increasing the secretion of digestion enzymes (Cahu and Zambonino-Infante, 1998), and changes in environmental parameters such as light regime (Naas et al., 1992). Reitan et al. (1993) found that the nutritional quality of rotifers decreased more rapidly in clear versus greenwater culture systems and that, over time, the fatty acid profile of the rotifers began to reflect that of the microalgae added to the systems. In this study, the growth and survival of cobia larvae fed live prey enriched with *I. galbana* was significantly greater than larvae fed prey enriched with *N. oculata*. If the nutritional properties of the live prey were significantly altered by the presence of algae in the rearing tanks, you would expect to see a difference in growth between the Algae-I and Algae-N treatments. Cobia larvae were pulse fed rotifers and *Artemia* throughout the day and very few, if any, were left in the tanks the following morning. Although it is probable that live prey remaining in the tanks acquired some of the characteristics of the microalgae used in the greenwater culture, the apparent short resident time of rotifers and *Artemia* in the rearing tanks would suggest this was not a factor and that additional factors commonly associated with greenwater culture systems also influenced the results presented here.

This study provides additional information regarding the rearing of cobia larvae in recirculating aquaculture systems beyond those previously reported in the literature (see Hassler and Rainville, 1975 and Faulk and Holt, 2003). For instance, no differences in growth or survival (day 16) were detected when larvae were fed *N. oculata* or Algamac 2000 enriched rotifers followed by *Artemia* enriched with Algamac 2000, suggesting that enrichment during the period of *Artemia* feeding may be more important than during the short rotifer feeding period. To our knowledge, this is the first study which shows that the survival of cobia larvae may be significantly improved by the addition of live algae (*I. galbana* or *N. oculata*) to the rearing tanks. This study also raises additional questions regarding the most favorable rearing conditions of cobia larvae in recirculating aquaculture systems. For instance, more detailed research is needed to clarify the requirement of cobia larvae for essential fatty acids during the rotifer and *Artemia* feeding periods in terms of growth, survival, and stress resistance. Further research is also needed to identify the

precise mechanisms responsible for increasing the survival of cobia larvae in greenwater culture and to evaluate the effect of different species and concentrations of microalgae. Current best management practices for cobia in recirculating aquaculture systems are to feed rotifers and *Artemia* enriched with *I. galbana* or commercial preparations, such as Algamac products, in a greenwater culture.

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