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Intensive larval husbandry and fingerling production of cobia *Rachycentron canadum*

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ABSTRACT

Methods and results of two larval rearing trials of cobia (*Rachycentron canadum*) are presented. These trials were designed to test the efficacy of protocols developed over several years of research in cobia larviculture at the UMEH. The protocols incorporate the use of probiotics and prophylaxis, minimize microalgae use, and include commercially available ingredients for live feed enrichment. During the trials, fertilized eggs were stocked at 400/L and incubated in 1000-L cylinder-conical tanks with flow-through seawater at 500% daily exchange rate. Moderate aeration and pure oxygen were used to maintain dissolved oxygen concentrations above saturation (6.5 mg/L at 26 °C). Hatching occurred at 22–24 h post fertilization. Two day-post-hatch (dph) yolk-sac larvae were stocked in four 12,000-L cylinder-conical tanks at 5 and 10 larvae/L. Beginning on 3 dph, larvae were fed microalgae (*Isochyrsis galbana* C-strain) at low concentrations (5–10,000 cell/ml) and enriched rotifers (*Brachionus plicatilis*) at 3–5/mL through 9 dph. Beginning on 7 dph, enriched Artemia (*Artemia franciscana* GSL Strain) nauplii were fed to larvae at 0.1–1/mL. Cobia larvae were reared at water temperatures ranging from 24.3 to 31.8 °C. Water quality parameters were within normal ranges for seawater: salinity 26–34 ppt, pH 7.92–8.16, and $NH_3 < 0.18$ mg/L. Vigorous aeration and supplemental oxygen were used at all times during both larval rearing trials to maintain adequate water movement and levels of dissolved oxygen (DO) (7.0–9.0 mg/L). Water was filtered down to 10 μm using standard sand filters filled with broken glass media and bag filters prior to entering the tanks. Daily water exchange rates in the tanks ranged from 100% at 3 dph to 500% from 17 dph onwards. Between 20 and 22 dph, all post-larvae were fully weaned onto dry starting diets. Survival rates of post-larvae measuring 1.5–2.0 cm SL and weighing 0.5 g at 20–22 dph were estimated to be ≥50%. Further mortality during the nursery stage to 3–5 cm and 1–3 g fingerlings prior to shipping at 27 dph brought the overall survival rate to an average of 25.7%. Survival rates of fingerlings cultured in tanks initially stocked at lower densities (5 larvae/L) was significantly higher ($P = 0.0078$). From 15 dph, post-larvae and fingerlings were daily graded by size with large individuals singled out and stocked into another tank. These trials generated 125,328 fingerlings in four tanks in just two months, levels of production that could sustain a commercial operation and indicate that cobia aquaculture can be viable in the Americas.

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1. Introduction

Cobia (*Racycentron canadum*) is the only member of the family Rachycentridae. It is a tropical and subtropical species widely distributed worldwide (Briggs, 1960; Shaffer and Nakamura, 1989; Ditty and Shaw, 1992), except in the eastern Pacific, where it occurs only rarely (Fowler, 1944; Briggs, 1960; Collette, 1999). Commercial aquaculture production of cobia began in southeast Asia in the late 1990s (Chang et al., 1999; Su et al., 2000; Liao et al., 2004; Nakamura, 2007). Efforts to conduct cobia larviculture in the United States date back to the mid 1970s (Hassler and Rainville, 1975) and continue today (Benetti et al., 2007; Holt et al., 2007).

Cobia is widely recognized as an excellent aquaculture species (Liao et al., 2004; Benetti et al., 2007). The fast growth rate of cobia led

to its initial recognition as a potential candidate for aquaculture (Hassler and Rainville, 1975). Since then, other traits such as high fecundity and ease of induced and natural spawning in captivity (Franks et al., 2001; Arnold et al., 2002) and adaptability to confinement in tanks and cages (Schwarz, 2004) have been identified and serve to reinforce the early conclusions about cobia. Liao et al. (2004) has summarized the success of cobia aquaculture in Taiwan. During the last decade, several Asian countries have begun raising cobia commercially, and the industry is developing fast in tropical and subtropical regions throughout the world. Most recently, Australia and the Marshall Islands have begun developing cobia aquaculture and it is also expanding in the Americas and the Caribbean. Current commercial project locations include the United States, Puerto Rico, the Bahamas, Belize, the Dominican Republic, Mexico, Panama, and Brazil (Benetti et al., 2007; Holt et al., 2007).

Despite the expanding interest in cobia aquaculture, many questions about larval growth and development remain unanswered

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and recent research efforts have focused on this area. Faulk and Holt (2003) examined the fatty acid composition of cobia eggs in order to gain insight into the nutritional needs of cobia during the first three weeks of their life and tested different larval feeding regimens in order to optimize survival and growth. Faulk and Holt also studied the comparative value of different live feed enrichment techniques in cobia larviculture (2005) and the tolerance of larval cobia to changing salinity levels (2006). In a complementary study, Faulk et al. (2007a,b) reported on the ontongeny of the cobia gastrointestinal tract and digestive enzymes during the first three weeks post hatch. Finally, Hitzfelder et al. (2006) studied the effect of stocking density on larvae growth and survival in recirculating aquaculture systems.

While this research has resulted in an improved state of the art for cobia larviculture, fingerling production is still an industry wide bottleneck and there remains a need for reliable hatchery technologies and protocols that will improve survival and increase production. The UMEH has been working to meet these needs since 2004 (Benetti et al., 2007), and this paper represents the culmination of those efforts to date.

2. Materials and methods

2.1. Egg incubation

Eggs were obtained from broodstock held at the UMEH in two 80,000-L maturation tanks. After the spawning events, floating eggs were collected from the two 80,000-L maturation tanks stocked with broodstock cobia using surface skimmers connected to 500-L tanks used as egg collectors via an overflow pipes in each maturation tank. Eggs were scooped from the egg collectors using a cotton net and placed in clean 20-L buckets and acclimated to ambient water temperatures (26±1 °C and salinity (32± 1 ppt) in the incubators. After the eggs had been acclimated (approximately 1 h post-fertilization), they were allowed to separate, with viable, positively buoyant eggs rising to the surface and non-viable, negatively buoyant eggs sinking to the bottom. At this point, the eggs were counted using previously published densities of 420 eggs/mL (Kaiser and Holt, 2005) and fertilization rates were calculated. Non viable eggs were removed from the 20-L buckets with a small siphon and discarded.

The fertilized eggs were then stocked at 400 eggs/L in two 1000-L cylinder-conical tanks supplied with filtered seawater, gentle aeration from an air ring on a central standpipe, and trickling pure oxygen from a single air stone. The incubators were fitted with 500 μm mesh on the standpipes to facilitate water exchange. Surface skimmers were used to remove accumulated organic material. Dissolved oxygen was maintained between 6.0 and 8.0 mg/L during incubation. Temperature in the incubators ranged from 26 to 31 °C. Photoperiod was natural and illumination was reduced by the 80% shade cloth integrated into the hatchery roof.

After stocking the incubators, eggs were sampled regularly to monitor early cell division, development, and egg viability. After approximately 12 h, eggs were disinfected in the incubators using formalin (37% formaldehyde solution) at 100 ppm for 1 h (Tamaru et al., 1999). During disinfection, aeration and oxygen supply were maintained but water exchange was suspended. After 1 h water exchange was restored to approximately 500%/day in order to flush residual formalin from the tank.

Hatch occurred 22–24 h after fertilization. After hatching, a volumetric sample was taken from the incubator to determine the total number of yolk-sac larvae and the attendant hatch rates. Once hatch rates were calculated, water supply to the incubator was suspended and a gentle circular current resulting in a vortex was created within the incubator. Over a period of 5–10 min, this vortex accumulated and deposited all the unhatched egg shells and non-viable eggs in the bottom of the tank where they were removed using a small siphon. Viable yolk-sac larvae are positively buoyant due to their large yolk-sac, and therefore remained suspended in the water column near the surface and were unaffected by this process. Removing debris from the incubator is critical as it eliminates a source of nutrient enrichment that would otherwise encourage bacterial colonization of the incubators.

Incubation continued until 2 dph. During this time incubator set-up remained as described above. Siphoning was also repeated at 2 dph as described in the previous paragraph. At the end of 2 dph, the yolk-sac larvae were collected and transferred by hand into the larval rearing tanks using 5-L transfer beakers.

2.2. Larval culture

Two larval rearing trials were conducted at UMEH during 2007, using four 12,000-L tanks with two replicate tanks per treatment. Natural photoperiod was used for both trails, with illumination reduced by the 80% shade cloth integrated into the hatchery roof. Salinity ranged between 32 and 35 ppt in both trials. In trial 1 (T1), water temperature ranged between 24.3 to 28.8 °C and in trial 2 (T2) it ranged from 29.4 to 31.8 °C. Two day-post-hatch (dph) yolk-sac larvae were stocked in four 12,000-L cylinder-conical tanks with central drains and standpipes fitted with 150–500 μm screens. During T1, probiotics were incorporated into the live

feeds (rotifers and Artemia) to test its effect on larval survival and growth. In T1, approximately 225,000 cobia yolk-sac larvae were split into two groups and evenly stocked in two 12,000-L larval rearing tanks (LRTs). One group of larvae received live feeds that had been treated with probiotic inoculates while the other group of larvae received only untreated feed. Stocking density was 10 larvae/L in both tanks. At 12 dph, each of these groups of larvae were graded and split into two additional 12,000-L tanks. The second trial (T2) was intended to test the effect of differing stocking densities on larval survival and growth. In T2, a total of 360,000 larvae were stocked in four 12,000-L tanks. LRT 1 and LRT 2 were stocked with 120,000 larvae each, a density of 10 larvae/L. LRT 3 and LRT 4 were stocked with 60,000 larvae each, a density of 5 larvae/L.

In both T1 and T2 cobia larvae were fed enriched rotifers (Brachionus plicatilis) using a pulse feeding technique, meaning that rotifers were added to the LRTs every 3 to 4 h to maintain concentrations of 5 rotifers/mL. The first feeding of the day was at sunrise and the final feeding was 1 h before sunset. Once the cobia had grown to a sufficient size, overlapping or co-feeding with artemia (Artemia franciscana GSL Strain) began. At the end of the co-feeding process, the cobia larvae were fed exclusively artemia. As with rotifers, artemia was provided to the LRTs using a pulse feeding method, with the first feeding just after sunrise and the last feeding about 1 h before sunset.

In T1, the larvae were fed enriched rotifers from 3 to 9 dph. Co-feeding occurred at 9 dph, with the larvae being provided both rotifers and enriched instar-2 artemia. For co-feeding, rotifers were supplied to the LRTs at a reduced density of approximately 2 rotifers/mL and artemia was provided at 0.4 artemia/mL. By 10 dph, larvae were being fed only enriched instar-2 artemia which was being put into the LRTs at a rate of 0.4–1.0 artemia/mL.

In T2, the larvae were fed enriched rotifers from 3 until 8 dph. Overlapping of rotifers and artemia was anticipated and lasted for a period of 3 days. At 6 dph, the co-feeding process began by feeding small amounts of freshly hatched instar-1 artemia (0.1 artemia/mL) to the cobia larvae along with the standard dosage of rotifers. At 7 and 8 dph, enriched instar-2 artemia were being provided to the LRTs at approximate densities of 0.2–0.3 artemia/mL, while rotifer feeding rates were reduced to 2.5–4 rotifers/mL. By 9 dph, the larvae were completely transitioned to enriched instar-2 artemia which was being fed to the tanks at a rate of 0.4–0.8 artemia/mL. In T2, the more densely stocked LRTs at 10 larvae/L (LRTs 3 and 4) were fed at the upper end of this range (0.5–0.8 artemia/mL) while the less densely stocked LRTs at 5 larvae/L (LRTs 1 and 2) were fed at the lower end of this range (0.4–0.6 artemia/mL).

In T1 live microalgae was continuously added to the LRTs during daylight hours in order to maintain low concentrations of 5000 to 10,000 cells/mL. In T1, microalgae was used from first feeding at 3 dph until 20 dph. In T2, production shortfalls at the UMEH considerably limited the use of microalgae. Reduced densities of 2000 to 10,000 cells/mL were maintained, and microalgae use ended entirely after 12 dph. Isochrysis galbana C-Strain was the primary microalgae used during both trials, although Tetraselmis chuii was also used on occasion.

Rotifers were grown in a semi-continuous culture system and fed a dry diet consisting of bakers yeast combined with microalgae in concentrated or dry form at 0.3–1.0 g/10^6 rotifers/day. 1000-L rotifer cultures were grown to high densities (approximately 1500–2000 rotifers/mL) and then harvested over a period of 2 to 3 days, leaving just enough rotifers to restart the culture. Prior to use as live feed, rotifers were enriched in their culture tanks for 24 h. The enrichment formula was a mixture of commercial products designed to maximize the amount of highly and polyunsaturated fatty acid (HUFA/PUFA) levels bio-encapsulated within the rotifers. This mixture was fed at a dose of 3 g/10^6 rotifers and consisted of 45% baker's yeast (Saccaromyces cerevisiae), 2.5% Algamac Protein Plus, 42.5% Algamac 3050, 5% Algamac ARA, (Aquafauna Bio-Marine, Inc., P.O. Box 5, Hawthorne, California, 90250)[1], 2.5% Astraxanthin (Naturose) and 2.5% Nannochloropsis Instant Algae (Reed Mariculture, Inc., 520 McGlincy Lane #1, Campbell, CA 95008). Enrichment ingredients were mixed with 15 L of fresh water, stored in a clean plastic container, and delivered to the rotifer cultures via a peristaltic drip pump continuously over a period of 24 h. After enrichment, rotifers were harvested using a 64 μm harvester and washed for 30 to 45 min using filtered, UV treated seawater. Harvested rotifers were stored in clean coolers, chilled to approximately 10 °C and used as needed for up to 24 h. No live microalgae was used in the rotifer cultures.

Artemia was enriched for 12 h after hatching in 300-L enrichment cones. As with rotifers, the artemia enrichment was a mixture of commercial products designed to maximize the amount of HUFA/PUFAs delivered to the larvae via the artemia. The enrichment diet consisted of 85% Algamac 3050, 10% Algamac ARA, and 5% Astraxanthin (Aquafauna Bio-Marine, Inc., P.O. Box 5, Hawthorne California, 90250) and was fed to the artemia at a rate of 0.3 g/10^6 artemia. Artemia enrichment was mixed with 3 L of water and added directly to the enrichment cones. Artemia were harvested using a 150 μm harvester and washed for 30 to 45 min with filtered, UV treated seawater. Like rotifers, harvested artemia were stored in clean coolers, chilled to approximately 10 °C, and used as needed for up to 24 h.

During T1, EcoPro probiotic inoculates (EcoMicrobials, LLC, 7003 North Waterway Drive, Unit 214, Miami, FL 33155) were introduced into one of the two rotifer cultures

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[1] Trade and manufacturer's names are used throughout this article in order to accurately and completely describe the materials and methods used during these trials. In no case does the use of trade or manufacturer's names imply endorsement.

and one of the two artemia hatching cones. Rotifers and artemia treated with probiotics were fed exclusively to one group of larvae while the other group of larvae received only untreated feed in an attempt to determine the effect of probiotics on larval survival. During T2, probiotic inoculates were used in all live feed production and enrichment and were fed to all larvae.

Probiotic dosage rates were consistent throughout T1 and T2. Inoculants of 10^3 CFU (Colony Forming Unit)/mL (1 mL of probiotics for every 100 L of culture water) were added directly to the rotifer culture tanks. Inoculants of 10^5 CFU/mL (1 mL of probiotics for every 1 L of water in the feed vessel) were added directly to the rotifer feed vessel. Artemia hatching and enrichment cultures received inoculants of 10^4 CFU/mL (1 mL of probiotics for every 10 L of culture water).

Along with probiotics, prophylactic formalin treatments were used on a regular basis. The formalin treatment protocol involved a multi-step process. First, water exchange in the tanks was secured and 50 ppm formalin was added to the tank. After 30 min, an additional 25 ppm formalin was added to the tank and static water conditions in the tank were maintained. After 1 h, water exchange was restarted and set to 500% for 2 h.

Finally, strict sanitary practices were followed throughout the course of both T1 and T2. Beginning at 2 dph tank bottoms were siphoned daily to remove dead larvae, uneaten feed, and other organic debris. Additionally, surface skimmers were cleaned with fresh water and paper towels and tank walls were wiped down at the waterline several times a day. Tank standpipes were pulled and thoroughly rinsed once a day, and by 10 dph were removed and cleaned twice daily.

2.3. Weaning

In both trials, weaning was initiated by introducing very small quantities of Otohime Weaning Diet B_1 (200–360 μm) (Marubeni Nisshin Feed Co., Ltd.) into the tank approximately 15 min prior to feeding artemia. Aeration was suspended when the dry diet was introduced into the LRTs. This allowed cobia larvae to swim to the surface to consume the floating feed and reinforced the desired feeding behaviors. After offering the dry diet, aeration was restored and artemia were fed to the larvae. Dry feed was offered to the cobia larvae six to ten times daily. The first feeding of the day was always at sunrise and the final feeding occurred no more than 1 h prior to sunset.

In T2, water temperatures ranged between 29.4 and 31.8 °C, and early weaning started at 11 dph and progressed fast with the amount of Artemia remaining essentially unchanged while the amount of starting dry diets increased exponentially. At 15 dph, feeding rates were 25 to 50 g/tank/day. At 17 dph the LRTs were receiving 50–100 g/tank/day. At 21 dph the post-larvae were completely weaned onto dry diets and the LRTs were receiving 170 g/tank/day. Throughout the weaning period, the less densely stocked tanks (LRT 3 and 4) received slightly less dry feed than the more densely stocked tanks (LRT 1 and 2). As the larvae developed and mouth gapes increased, progressively larger feeds were used. At 19 dph Otohime B_2 (360–620 μm) was introduced and at 22 dph Otohime C_1 (920–1410 μm) was being used. The switch from one feed size to another was always an incremental process, with one feed gradually replacing another over a period of two or three days. The decision to completely suspend the use of live feeds was based largely on observation of the post-larvae feeding behavior.

In T1, temperature ranged from 24.3 to 28.8 °C, and weaning began at 19 dph in all tanks with Otohime B1. By 22 dph the LRTs were receiving 50 g of Otohime B_1 and B_2 daily. At 25 dph the post-larvae were completely weaned onto dry-diet and the LRTs were receiving 200 g of Otohime B_2 and C_1 daily. Finally, size grading the post-larvae and early fingerlings in both trials began as early as at 12 dph and continued throughout the entire weaning process. Grading is a tedious process: larger larvae were sorted out and scooped out of the tanks several times a day using a 3-L plastic beaker and transferred to another tank maintained solely for the purpose of holding the very large post-larvae/early fingerling. When they were considered large enough to be handled by nets, they were netted and transferred to another tank.

2.4. Water quality

In both trials, all tanks (including isolation tanks and incubators) were set up to run on a flow through basis. Incoming seawater was glass media filtered and then passed through a 10 μm sock filter prior to entering the tank. UV filtration was not used except in rotifer and artemia culture systems. Water quality was sampled on a regular basis to ensure that parameters stayed within the range of values normally associated with seawater. Salinity remained between 26 and 34 ppt, pH stayed within a narrow range between 7.92 and 8.16, and NH_3 concentrations never rose above 0.18 mg/L. In T1 water temperature ranged from 24.3 to 28.8 °C. During T2 water temperature was between 29.4 and 31.8 °C.

Water exchange rates were progressively increased throughout the course of both T1 and T2. At 2 dph, water exchange in the tanks was set at 100%/day during daylight hours and increased to 200%/day during the evening. At 7 dph water exchange was increased to 200% during daylight hours and 300%/day during the evening. By 17 dph water exchange was set to 400–500%/day throughout the day. The LRTs were initially fitted with standpipes covered with 300 μm mesh. In order to facilitate the increasing water exchange rates the mesh size was increased to 500 μm at 8 dph and to 1000 μm at 17 dph.

Aeration was also increased progressively during both trials. At 2 dph moderate aeration was provided as well as supplemental oxygen. By 17 dph, vigorous aeration was being provided to all tanks in order to reduce cannibalism, ensure a more even distribution of the larvae during the weaning process and maintain DO levels between 7.0 and 9.0 mg/L.

2.5. Statistical analysis

Data obtained from egg incubation and larval rearing during T1 and T2 were analyzed for normality and statistical differences in variance. All data were analyzed using Excel version 2007 (Microsoft, One Microsoft Way, Redmond, WA 98052–6399) and Stata Release 10 (StataCorp LP, 4905 Lakeway Dr., College Station, TX 77845). Data found to be normally distributed was analyzed for variance then checked for significant differences within treatments using t-tests to a significance level $\alpha = 0.05$. Data were then pooled, analyzed for normality and variance, and significant differences between treatments were analyzed using t-tests to a significance level $\alpha = 0.05$. Percent survival data obtained from the different larval rearing trials were first angular (arcsine) transformed before being analyzed for statistical differences in variance, and then analyzed via a t-test to a significance level of $\alpha = 0.05$. Raw length growth data was analyzed for normality and differences in variance, then analyzed utilizing t-tests or non-parametric Kolomogorov–Smirnov tests to a significance level of $\alpha = 0.05$ for differences within treatments. Raw length data was then pooled, analyzed for normality and differences in variance, then tested utilizing a t-test to a significance level of $\alpha = 0.05$ in order to detect differences between treatments.

3. Results and discussion

3.1. Spawning and larval culture

In T1, 225,000 yolk-sac larvae were initially stocked in two 12,000-L larval rearing tanks resulting in the production of 41,470 fingerlings. In T2, 360,000 yolk-sac larvae were stocked in four 12,000-L larval rearing tanks resulting in the production of 83,858 fingerlings (Table 1). Statistical analysis of data collected during T2 revealed that different stocking densities (5 larvae/L and 10 larvae/L) led to significantly different larval survivorship and growth rates, but did not significantly affect final density (Table 2). Final survivorship to day 27 in the two higher density tanks was 19.2 and 17.5% respectively, while survivorship in the two low density tanks was 31.4 and 34.9% (t-test, equal variance, $P = 0.0078$). Mean Total length (TL) at 21 dph in the higher density tanks was 18.85 mm and 21.29 mm, while TL at 21 dph in the lower density tanks was 24.72 mm and 27.48 mm indicating that larvae reared at lower densities grew significantly faster than their counterparts reared at a higher density (t-test, equal variance, $P = 0.000$) (Tables 1 and 2, Fig. 1). Additionally, an analysis of the size frequency distribution between the two treatments revealed that relatively more large larvae were found in the low density LRTs (5 larvae/L) than in the high density LRTs (10 larvae/L) (Fig. 1). In the two high density tanks, final stocking density at 27 dph was 1.92 and 1.75 larvae/L respectively and in the two low density tanks the final stocking density was 1.57 and 1.74 larvae/L, a difference that was statistically insignificant (t-test, equal variance, $P = 0.15$) (Table 2).

These results are generally in agreement with recently published data on cobia larviculture. Hitzfelder et al. (2006) studied the effects of initial stocking density on cobia larval survivorship, growth rates, and final density. While they did not report a significant difference in final larval survivorship between treatments with initial stocking densities of 5 and 10 larvae/L (as was shown in T2), the trials did reveal a general trend towards lower survivorship and growth at higher stocking densities (above 10 larvae/L). Further, as in T2, Hitzfelder et al. (2006) did not report significant differences in final stocking densities when comparing initial stocking densities of 5 and 10 larvae/L. In a different series of studies on cobia larviculture at the University of Texas Marine Science Institute, researchers were able to grow cobia to 1.47 cm by 22 dph (Faulk et al., 2007a,b) and to 4 to 5 cm by 30 dph and achieved survival rates as high as 24.5% (Holt et al., 2007). Larviculture trials conducted in ponds in Taiwan have also resulted in high growth rates, with cobia larvae growing to as much as 8–10 cm by 45 dph (Liao et al., 2004). To the authors' knowledge, however, the survival rates to 27 dph (19.2 to 34.9%) and overall fingerling production (83,858 fingerlings) achieved in four 12,000-L tanks at the UMEH during T2 are the highest ever reported for a single trial (Table 3).

A combination of techniques from previous larval rearing trials conducted at the UMEH that were used in this trial likely contributed to the high rates of survival achieved during these two trials. First, the live feed enrichment protocol ensured that high levels of crucial HUFA/PUFA lipids such as docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and arachidonic acid (ARA) were consistently delivered to the cobia larvae. It is well established that larval marine fish exhibit higher survival

Table 1
Summary of total yield, survival, and mean final total length from T2

Tank	Stocking density	Final production	Survival %	Mean final TL (mm) 21 DPH ($N = 30$)
LRT1	10 larvae/L (120,000)	23,061	19.2	18.85
LRT2	10 larvae/L (120,000)	21,001	17.5	21.29
LRT3	5 larvae/L (60,000)	18,836	31.4	24.72
LRT4	5 larvae/L (60,000)	20,960	34.9	27.48
TOTAL	360,000 larvae stocked	83,858	23.3	N/A

Yield and survival were determined during final grading and shipping of the produced fingerlings, which lasted from 21 DPH to 27 DPH.

Table 2
Summary of results of statistical results from T2

Parameter	Test	P value	Significance
Length 15 DPH (pooled within treatment)	t-test, equal variance	0.000	Significant
Length 21 DPH (pooled within treatment)	t-test, equal variance	0.000	Significant
Survivorship (arcsine transformed)	t-test, equal variance	0.0078	Significant
Final juvenile yield	t-test, equal variance	0.15	Not Significant

and growth rates and resistance to stress when these nutrients are provided in sufficient amounts (Mourente et al., 1993; Koven et al., 2001; Faulk and Holt, 2003). Many of the products (especially Reed Mariculture's Nannochloropsis Instant Algae and Aquafauna BioMarine's Algamac Protien Plus, 3050 Flake, and ARA) utilized in the enrichment protocol were chosen specifically because they contained high levels of one or more of these essential fatty acids. Further, the practice of storing harvested enriched live feeds at approximately 10 °C ensured that the rotifers and artemia retained a large amount of the stored lipids until they could be fed to the larvae (Rainuzzo et al., 1989; Sorgeloos et al., 2001). Additionally, the use of formalin treatment as prophylaxis throughout the entire course of the larval rearing probably helped to prevent disease outbreaks and improve larval survival. Formalin was administered whenever microscopic analysis of the fish revealed any signs of bacterial or parasitic infestations that could compromise the larvae. Microscopic examination focused largely on the finfold and on the gills of the larval fish when formed. Whenever the finfold was observed to show any sign of deterioration during the daily samples, a formalin treatment was applied. The efficacy of formalin against viral, bacterial, and fungal pathogens of both marine and freshwater fish has been widely reported (Schreier et al., 1996; Rach et al., 1997; Tamaru et al., 1999). In past larval rearing runs at the UMEH, prophylactic formalin treatments were used much less frequently and losses from outbreaks of the dinoflagellate ectoparasisite Amyloodinium ocellatum, and the bacteria Photobacterium damselae were much higher than those previously experienced (Benetti et al., 2007, 2008).

Probiotic based disease prevention techniques for aquaculture have been in development since the 1980s (Farzanfar, 2006) and have, in recent years, begun to show promise (Douillet and Langdon, 1994; Carnevali et al., 2004; Rotman et al., 2005). Gatesoupe (1999) provided a thorough review on the use of probiotics in live feeds for marine fish larviculture and aquaculture in general. Although not evident from the results of T1, it is also possible that consistent use of probiotics in live feeds fed to the larvae contributed to the high production levels seen during T2. During T2, larval cultures were provided with consistent dosages of probiotics until weaning onto commercially prepared dry-diets was complete. The UMEH is continuing to conduct probiotic research in conjunction with EcoMicrobials LLC in an attempt to generate a more complete understanding of the effects of probiotics in marine fish larval culture systems.

The use of microalgae in larviculture of marine fish is recognized as extremely important from the environmental, nutritional and economic perspectives. Interestingly, the high levels of production in T2 were achieved despite limited use of greenwater. Microalgae (I. galbana) was only maintained at 5000–10,000 cells/mL in the LRTs for the first 12 dph, and was then eliminated altogether. This seems to contradict many of the previous studies that indicate that adding large amounts of live microalgae to culture tanks significantly improves the survival and growth rates of a wide range of larval marine fish (Nass et al., 1992; Reitan et al., 1998; Papandroulakis et al., 2002), including cobia (Faulk and Holt, 2005). Questions remain about the role of microalgae on survival and growth of cobia larvae, and further studies should address this important issue.

As larvae utilized for T1 and T2 came from two different broodstock sources at the UMEH, any attempt to assess the potential effect of egg quality on survival rates and overall results of the larval rearing trials would be speculative. For this reason, the trials were described and statistically analyzed separately and only compared when appropriate. T1 utilized eggs collected from a group of 12 wild broodstock fish kept in a 80,000-L recirculating system, four of whom had been injected with 1000 IU of HCG per kg of body weight two days prior to spawning. The spawn occurred on May 12, 2007; 1,473,900 eggs were produced in this spawn, with fertilization rate of 48.30% and hatch rate of 54.70%. The final average survival rate of the 225,000 yolk-sac larvae taken from this batch of eggs for use in T1 was 18.44%. This group of wild broodstock fish did not spawn again during 2007. T2 utilized eggs collected from a natural spawn from a group of eleven F1 broodstock fish kept in an 80,000-L recirculating system. The spawn
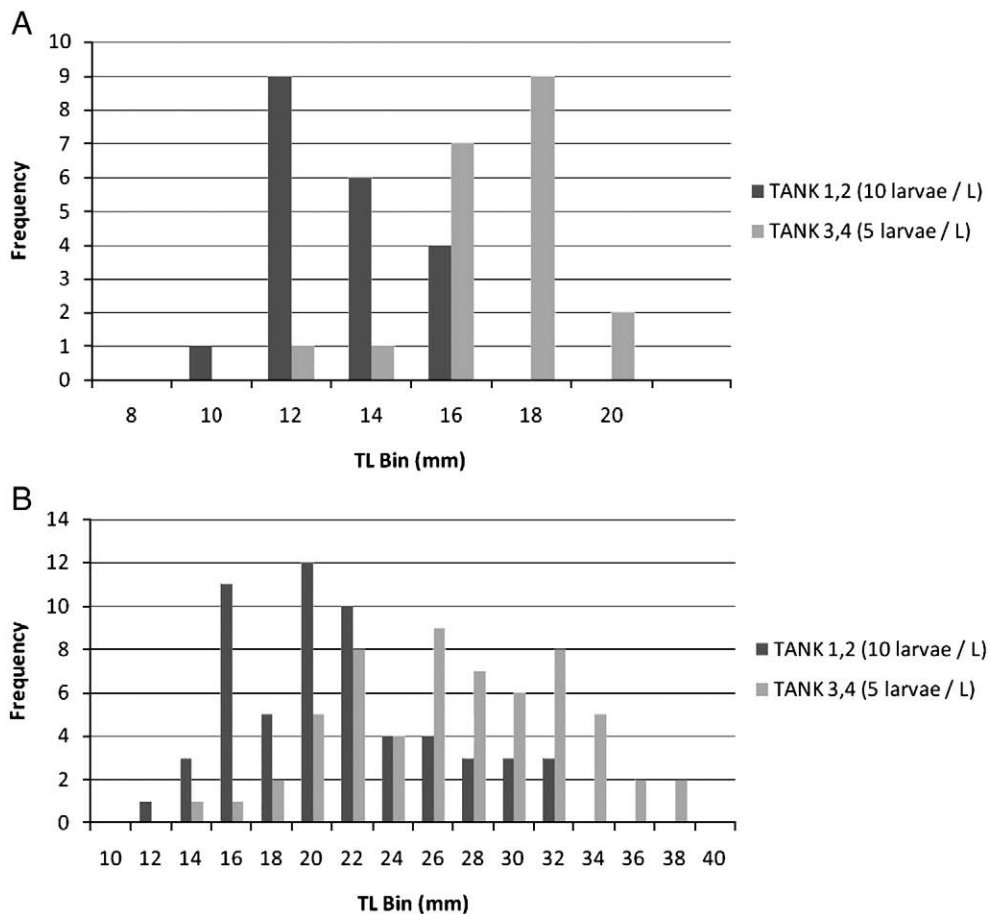
Fig. 1. Frequency distributions of total length of larvae, pooled within treatment at A) 15 DPH; and B) 22 DPH. * Note: Differences in size distributions between stocking densities are readily observed.

Table 3
Summary of data available on larval culture of cobia including survival rates, average final length, and growth rates

Culture system	Initial stocking density	Temperature (°C)	Survival (%)	Average final length (mm)	Reference
Intensive flow through (tanks)	5 larvae/L	29.4–31.8	31.4–34.9	26.1±5.6 (21 dph)	Current study, T2
Intensive flow through (tanks)	10 larvae/L	29.4–31.8	17.5–19.2	20.4±5.6 (21 dph)	Current study, T2
Intensive, recirculating (tanks)	10 lar/L	28.0–29.5	21.8–28.2	N/A	Holt et al. (2007)
Intensive, recirculating (tanks)	4 eggs/L	25–26	N/A	40–50 (30 dph)	Holt et al. (2007)
Intensive, recirculating (tanks)	N/A	28.5	N/A	67 (32 dph)	Holt et al. (2007)
Intensive, recirculating (tanks)	8.7 larvae/L	27.4±0.5	13.2	14.7 (22 dph)	Faulk et al. (2007a,b)
Intensive, recirculating (tanks)	14.7 larvae/L	27.5±0.4	10.4	14.6 (22 dph)	Faulk et al. (2007a,b)
Intensive, recirculating (tanks)	5 larvae/L	27	12.7±0.9	13.9±0.5 (21 dph)	Hitzfelder et al. (2006)
Intensive, recirculating (tanks)	10 larvae/L	27	9.4±0.7	14.1±0.5 (21 dph)	Hitzfelder et al. (2006)
Semi-intensive (ponds)	0.06 larvae/L	26.5–30.7	5.3–8.5	124.1 (35 dph)	Weirich et al. (2004)
Semi-intensive (ponds)	N/A	22–31	5–10	80–100 (45 dph)	Liao et al. (2004)

occurred July 3, 2007; 970,200 eggs were produced in this spawn, with fertilization rate of 95.02% and hatch rate of 74.97%. The overall survival rate of the 360,000 yolk-sac larvae taken from this batch of eggs for use in T2 was 23.3%. These F1 broodstock began spawning on May 6, 2007 and continued throughout the summer, finishing on September 17, 2007. A total of 11 natural spawns were recorded during this period, producing an average of 1,210,000 eggs, with fertilization rates of 94.58% and hatch rates of 81.28%. This was the first spawning season for these fish. Broodstock conditioning and spawning at UMEH are described in detail by Benetti et al. (2008).

3.2. Metamorphosis and weaning

The switchover from live feeds to inert, dry starting diets is a complex physiological process that is inexorably associated with a critical period of mortality in marine fish larvae/post-larvae. Mass mortalities are often observed during the weaning period, as post-larval and early fingerling fish appear most susceptible to disease outbreaks at this stage. Biotic characteristics such as rates of metabolism and abiotic factors such as temperature dictate when weaning should occur in marine fish (Benetti, 2002). The process of transitioning cobia post-larvae from live feeds to commercially formulated dry-diets seems to be primarily dependent on water temperature. At higher temperatures (>29 °C), the larvae grew faster, underwent metamorphosis much earlier, and were capable of transitioning to starting diets quicker than larvae reared at lower temperatures. Therefore, it is important to tailor weaning schedules to water temperature and the associated growth rates. At temperatures above 29 °C, as it was the case in T2, weaning may begin as early as 11 dph, and research on the ontogeny of the cobia digestive tract suggests that larvae may even be capable of taking commercially prepared dry-diets by 8 dph (Faulk et al., 2007a). At temperatures below 29 °C, however, weaning may have to be delayed until as late as 16 dph.

In T2, warmer temperatures (29.4–31.8 °C) accelerated the weaning process relative to T1 and to previous larval rearing trials at the UMEH (Benetti et al., 2007). Gill primary lamellae formation along with red blood cells were observed in the cobia larvae at 9 dph, indicating the onset of metamorphosis. Shortly thereafter, at 11 dph, weaning the larvae onto dry diets began. As weaning progressed, the amount of artemia offered remained essentially static and the increasing nutritional and caloric needs of the larvae were met by offering larger and larger amounts of dry feed. In T1, lower water temperatures (24.3 to 28.8 °C) resulted in slower gill lamellae formation and later onset of metamorphosis than in T2. Accordingly, the weaning process in T1 proceeded less rapidly than in T2. Nonetheless, in T1, weaning began at 19 dph in all tanks with Otohime B₁. The weaning process is gradual in nature, and our results suggest that it should take at least four to six days for cobia larvae. While more research is needed to identify the precise amount of time needed to successfully wean cobia onto commercially prepared dry-diets, relatively prolonged weaning periods have been reported by other researchers involved in cobia larviculture (Liao et al., 2001; Holt et al., 2007) and appear to be generally beneficial for most types of marine fish larviculture (Rosenlund et al., 1997).

Sanitary practices become much more important during weaning due to the increased biomass in the LRTs. Larger post-larval fish generate greater amounts of metabolic by-products, and the large amount of commercially formulated dry-diet used means that uneaten feed will inevitably accumulate on tank bottoms. Every item of equipment used inside the LRTs was cleaned and disinfected at least once a day, and protein skimmers were cleaned several times a day. Spot siphoning of the LRTs to remove mortality or biofouling of tanks began as soon as larvae were stocked, and complete siphoning of the bottom of the tanks was daily conducted beginning at 6 dph.

3.3. Diseases and other constraints

Despite their relative hardiness, cobia are susceptible to a wide range of pathogens, including viruses (such as Lymphocystis), bacteria (such as *Vibrio*), and various types of parasites (such as myxosporidea and *A. ocellatum*) (Liao et al., 2004). At the UMEH, larval cobia have proven to be particularly vulnerable to outbreaks of bacterial enteritis, bacterial gill disease and Amyloodinium (Benetti et al., 2007). As discussed above, there were no major outbreaks of diseases of any sort during either T1 or T2. Mortality in all tanks appeared to be the result of natural causes, the

increasing metabolic demands associated with growth during early developmental stages (Feeley et al., 2007), the physiological stress caused by the switch from cutaneous to gill respiration (Benetti, 1992), and the problems associated with high density culture conditions, such as competition for feed and cannibalism (Faulk et al., 2007a,b). Mortality levels increased markedly in all LRTs during both T1 and T2 after the onset of metamorphosis and the beginning of the weaning process and ceased near the end of the fourth week post hatch. During T1, significant mortality (more than 100 larvae/day/tank) occurred at 2 and 3 dph. Mortality then returned and stabilized at negligible levels until 15 dph. At 16 dph, mortality spiked due to rapidly dropping dissolved oxygen levels to <4.0 mg/L in all the LRTs necessitating the provision of pure oxygen into all tanks. After 16 dph, mortality remained above 1000 larvae/tank/day. Mortality during T1 had largely ceased by 28 dph. During T2, significant mortality did not begin to occur until 18 dph and increased rapidly thereafter. A spike of approximately 19,000 larval mortalities was observed at 22 dph. Mortality during T2 had largely ceased by 29 dph. Prophylaxis, adequate water quality in conjunction with adequate nutrition provided by appropriate enrichment and the consistent application of probiotics in live feeds likely played a key role in minimizing loss from previously reported troublesome diseases such as bacterial enteritis and *A. ocellatum* (Benetti et al., 2007).

A large size variation was observed during early larval development stages, with larger individuals growing faster through post-larval and juvenile stages. This size variation was exacerbated when larvae were reared at high densities. To avoid cannibalism, size grading was diligently conducted daily, with larger larvae and post-larvae single out and removed using soft cotton nets and stocked into another tank kept specifically for this purpose. In both trials, the tendency towards cannibalism among cobia post-larvae (Liao et al., 2001; Weirich et al., 2004; Faulk et al., 2007a,b) was exacerbated by the extreme size variation that began to develop during the third week post hatch. In T2, many of the larger post-larvae were more than twice the size of their smaller counterparts by 21 dph, and as expected the larger fish were constantly engaged in predatory behavior towards the smaller fish. Larger post-larvae often choked to death on smaller ones that they had attempted to consume. Similar patterns of predatory behavior were observed during T1. Higher mortality levels during the later stages of the larval rearing process required daily siphoning of the bottom of LRTs and to routine grading of the larvae by size. Siphoning dead post-larvae from tank bottoms at least once daily removes a potential vector for bacterial colonization of the tanks and regular grading helps maintain uniformity of size among the post-larvae which lowers the incidence of cannibalism. Finally, vigorous aeration helps to keep larvae and post-larvae constantly swimming and evenly distributed in the tank, helping to reduce cannibalism. The tendency towards cannibalism can be mitigated by the use of these techniques, but it does not appear possible to completely eliminate it.

Water quality can quickly deteriorate during larval rearing of fast growing species exhibiting high metabolic rates like cobia. Early in the larval rearing process, water quality was relatively easy to maintain by exchange rates of 100–300%/day. Biomass (even in densely stocked LRTs) was relatively low and, as indicated from an analysis of water quality data (particularly ammonia) metabolic wastes produced by the larvae were negligible. While microalgae assist in maintaining water quality in larval rearing tanks by uptaking nutrients, large quantities of rotifers and artemia can be detrimental. Uneaten rotifers and artemia continue to actively excrete metabolities in the water for long periods of time and should be flushed out of the tanks. This does not seem to represent a problem with cobia as larvae and post-larvae are voracious and consumed most if not all available live feeds within 1–2 h after they have been introduced into the tanks. As weaning progresses, however, water quality maintenance becomes much more difficult and important. Commercial starting diets tend to accumulate on tank bottoms and degrade water quality unless eaten or siphoned immediately. Regular measurements of total ammonia nitrate (TAN) during both T1 and T2 revealed that NH₃ concentrations rose as high as 0.18 mg/L at 18 dph. While the optimal TAN levels are not determined for larval cobia, it seems likely that levels much above those reported here will be detrimental to larval survival and growth, therefore we recommend that TAN be maintained below 0.2 mg/L. This required high flushing rates during the later stages of larval rearing and frequent siphoning. In these trials, water exchange rates were maintained at >300%/day and even >500% later stages, and the bottom of the tanks were siphoned twice a day.

Finally, we hypothesize that potential losses from previously identified outbreaks of diseases such as bacterial enteritis caused by *Photobacterium sp* and *Vibrio spp* and/or *A. ocellatum* were minimized and perhaps eliminated by the prophylactic treatments of formalin and the use of probiotics. Results suggest that hatchery production of cobia fingerlings should rely on improved live feeds enrichment and prophylactic protocols, use of probiotics in live feeds, reduced initial stocking densities to 5 larvae/L and continuous size grading.

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