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Can umbrella-stage *Artemia franciscana* substitute enriched rotifers for Cobia (*Rachycentron canadum*) fish larvae?

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ABSTRACT

Appropriate food of suitable nutritional value is crucial for first-feeding stages of the larvae of cobia *Rachycentron canadum*, a very fast growing marine fish species. Best survival and growth results in cobia larviculture have been reported with a starter diet of HUFA-enriched rotifers and –as mouth size permits – followed by freshly-hatched and eventually HUFA-enriched *Artemia* nauplii. Using the smaller-sized Vietnam *Artemia franciscana* (AF) strain instead of the Great Salt Lake *A. franciscana* strain, it has been shown that the rotifer-feeding period could be shortened with 3 days, resulting in significant improvements in larval survival and growth. This study verified the possibility to feed umbrella-stage *Artemia* for further shortening and eventually completely substituting rotifer start feeding.
The experiment was conducted in 200-L tanks and lasted 18 days. AF umbrella *Artemia* was used as sole feed during the whole rotifer feeding period (UAF), compared to the use of enriched rotifers for the first 2 days followed by AF-umbrella (ER+UAF) and the use of enriched rotifers as control (ER). The feeding incidence of UAF treatments was significantly lower ($P<0.05$) in the 1st feeding day, however, the ingestion and digestion of AF were evident. Growth and survival as well as deformities at day 18 post-hatching were not significantly different for all treatments ($P>0.05$). The viability of cobia larvae after exposure to high salinity stress was lower in the ER treatment at day 8 post-hatching, but higher at day 18 post-hatching ($P<0.05$). The ability of cobia larvae to ingest and digest AF umbrella at first feeding as well as the nutritional suitability of AF umbrella is discussed. The possibility to use umbrella-stage *Artemia* opens an opportunity to simplify the rearing protocol and to reduce production costs of cobia larviculture.

1. Introduction

The tropical marine fish cobia (*Rachycentron canadum* L., 1766) has a broad distribution, a high flesh quality and is a rapid growing species, making it an excellent candidate for mariculture (Chou et al., 2001; Liao et al., 2004). Although artificial reproduction of cobia has been successful since 1994 in Taiwan (Liao et al., 2001), the seed production has mainly been based on extensive larval rearing in ponds (Liao et al., 2004), often resulting in unpredictable quality and quantity. Intensive production in closed recirculating system on a diet of rotifers and *Artemia* followed by weaning onto formulated feeds has resulted in an average larval survival of 13.2% at day 29 post-hatching (Faulk et al., 2007a).

Nutrition at first feeding is a key factor affecting growth and survival in most marine fish species (Koven et al., 1999; Rainuzzo et al., 1997; Rønnestad et al., 1999, 2003). Rotifers, freshly-hatched *Artemia* nauplii followed by nauplii enriched with specific lipids and vitamins are a very classic feeding regime for many fish species (Dhert et al., 2001; Sorgeloos et al., 2001). In very fast growing species, such as cobia, the larval diet must suit both energetic as well as nutritional requirements. Faulk and Holt (2003) and Benetti et al. (2007) have shown that freshly-hatched *Artemia* nauplii of the smallest *Artemia franciscana* (AF) strain should be offered at an earlier larval development stage of cobia i.e. from day 5 after onset of exogenous feeding. In this study, we want to explore if further improvements can be expected by starting *Artemia* feeding still earlier through the use of pre-hatched, so-called umbrella-stage *Artemia*, eventually allowing the complete replacement of rotifers, a diet which is cumbersome to produce and prone to bacterial contamination (Battaglene et al., 2006; Øie et al., 1994; Vanhaecke et al., 1990).

Umbrella-stage *Artemia* can be collected during the hatching process of *Artemia* cysts, i.e. upon breaking of the cyst shell or chorion, the pre-nauplius larva, still surrounded by its hatching membrane protrudes from the shell and as a result of its specific buoyancy characteristics hangs underneath the empty shell, hence the name umbrella-stage. This cyst "breaking" process ends when the hatching membrane breaks and the free-swimming instar-I nauplius larva emerges (Fig. 1).

Among the *Artemia* strains recently used in aquaculture, *A. franciscana* produced in Vietnam (Clegg et al., 2000) has a small size and high eicosapentaenoic acid (EPA) content and was therefore selected to

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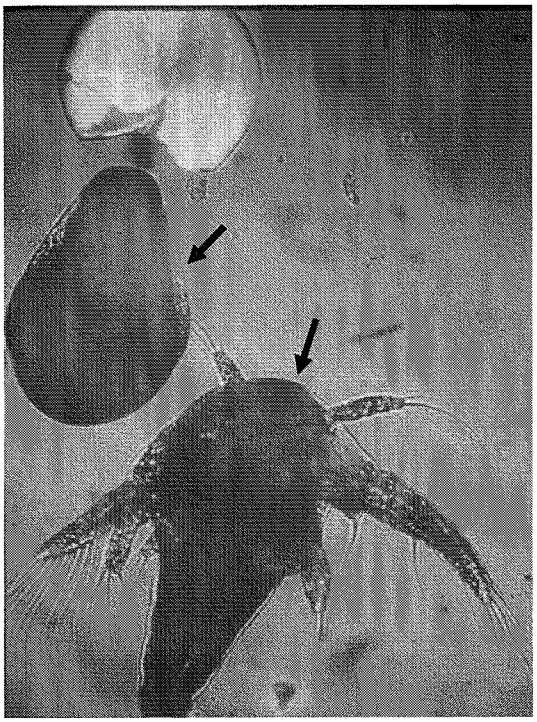

Fig. 1. Development of an *Artemia* cyst into umbrella-stage and instar I nauplius stage (Lavens and Sorgeloos, 1996).

produce umbrella for use as feed for the first feeding trial of cobia larvae. The idea of using AF umbrella, if successful, can open several opportunities: (1) simplify the larvae rearing protocol by eliminating the use of enriched rotifers; (2) alternative substitution feed in case of sudden crashes of rotifer production or shortage of rotifer production; (3) provide a more energy-rich food in comparison with enriched rotifers for the larvae during the development and (4) benefit from extra energy present in cysts that is lost during the hatching process and/or swimming activities of nauplii. The objectives of this research therefore, were to test the possibility of total or partial replacement of rotifers by using AF umbrella and evaluate its effects on growth and survival of cobia larvae at an early development stage.

2. Materials and methods

2.1. Experimental design

Three treatments were randomly allocated in nine 200-L fibre glass tanks (three replicates for each treatment) under indoor conditions at the Aquaculture Research Sub-Institute for North Central, Nghe-An Province, Vietnam. AF umbrella were used as sole food during the whole period (treatment 3 = UAF), compared to the use of enriched rotifers for the first 2 days followed by AF umbrella (treatment 2 = ER + UAF) and the use of enriched rotifers as control (treatment 1 = ER) (Table 1).

2.2. Live food preparation

Nannochloropsis oculata and *Isochrysis galbana* were cultured in 15-L plastic bags following standard protocols (Lavens and Sorgeloos, 1996) in natural seawater at 22 °C. The *N. oculata* was added to the larvae tanks, while *I. galbana* was used as food for the rotifers after enrichment.

Rotifers (*Brachionus plicatilis* sensu strictu) were cultured in 500-L tanks using Culture Selco Plus and enriched in separate tanks using Protein Selco Plus (INVE Aquaculture SA, Belgium) for about 11 to 15 h according to the manufacturer's protocol. After careful rinsing of the enriched rotifers with sea water on a 55-μm mesh, some were transferred to the larval rearing tanks, while the rest was stored for maximum 8 h in 30-L fibre glass tanks at 10–12 °C with *I. galbana* at a density of 1×10^7 cells mL^{-1} in order to maintain optimal nutritional quality.

AF-brand *Artemia* cysts (INVE Aquaculture SA, Belgium) were disinfected and incubated at 28–30 °C in 33 g L^{-1} seawater under continuous light and strong aeration (Lavens and Sorgeloos, 1996). After 10–12 h incubation, samples were taken to check the breaking stage of the cysts and the presence of umbrella. In case more than 50% of the cysts had reached umbrella stage, they were collected on 70-μm mesh, washed with seawater and stirred for 15 min in a 2-L beaker using a magnetic stirrer. The umbrellas were separated by sedimentation in a cylindro-conical bottle, while unhatched cysts and empty shells, floating at the surface, were collected and re-incubated. This procedure was repeated every hour and lasted for 2–3 h (depending on the cyst quality and water temperature). The collected umbrellas were either directly used or stored at 5–7 °C for maximum 5 days. Since *Artemia* umbrella sediment quickly, they were kept in suspension in

Table 1
Experimental feeding regime

Day after hatching	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
General rearing protocol (as applied for all treatments)																		
N. oculata																		
Nauplii of AF Artemia																		
Enriched EG Artemia																		
Dry feed NRD 2/3[a]																		
Treatment 1 (ER)																		
Enriched rotifers																		
Treatment 2 (ER + UAF)																		
Enriched rotifers																		
AF umbrella																		
Treatment 3 (UAF)																		
AF umbrella																		

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aerated plastic bottles installed above the larval fish tank from where they were added via a valve-operated dripping system.

EG-brand *Artemia* cysts (Great Salt Lake USA, INVE Aquaculture SA, Belgium) were disinfected and incubated as described above (Lavens and Sorgeloos, 1996). The nauplii were enriched with A1 DHA Selco (INVE Aquaculture SA, Belgium) for 24 h following the manufacturer's protocol.

2.3. Larval rearing

Newly-hatched cobia larvae obtained from captive broodstock fed experimental pellets (EWOS Ltd., Canada, containing 1.8% HUFA), were stocked at a density of 50 ind L^{-1} in 200-L cylindro-conical tanks equipped with aeration and light in an air-conditioned room. The photoperiod was set at 10 h:14 h (dark:light). All tanks were connected to a bio-filter (modified from Menasveta et al., 2001), a protein skimmer and UV treatment system to maintain stable water quality, i.e. salinity 33.0±0.5 g L^{-1}, DO 6.37±0.20 mg L^{-1}, water temperature 28.0±0.1 °C and pH 8.0–8.2. After 4 days post-hatching (dph), water was exchanged at an initial flow rate of 1 L min^{-1} and gradually increased up to 4 L min^{-1} by 12 dph. A 500-μm filter cylinder ensured removal of uneaten rotifers and *Artemia* umbrellas. The effluent water was drained over a 55-μm mesh followed by a 22-μm mesh filter to remove rotifers and *Artemia* before the inlet of the bio-filter. The removal of uneaten rotifers and *Artemia* and regularly replacement by the new ones, ensured high quality live food in the rearing tanks.

From 2 dph, *N. oculata* were added to the larval tanks at an algal density of 1×10^5 cells mL^{-1}. Enriched rotifers or AF umbrella (according to the different treatments) were added from 3 dph at a starting density of 5–7 ind mL^{-1} or 0.1 ind mL^{-1}, respectively and remained at a density of 10–12 ind mL^{-1} and 0.5–2 ind mL^{-1}, respectively, during the following days. Live food (algae, rotifers, *Artemia*) was added four times a day to keep the density constant. Environmental factors such as DO, pH, water temperature (OxyGuard Hundy Gama) and salinity (refractometer model FG-211) were inspected twice a day (7 am and 2 pm). Every 2 days, the bottom was siphoned to remove accumulated organic matter. The experiment was terminated after 18 days rearing.

2.4. Sampling and fatty acid analysis

The enriched rotifers and enriched nauplii of EG-brand *Artemia* were collected in the interval of feeding times (2 h after harvest from enrichment tanks) on a sieve of 55 μm (for rotifers) and 110 μm (for *Artemia* nauplii) and rinsed carefully with sea water, followed by extensive washing with distilled water. Excess water was removed by putting the net on tissue paper. Subsequently, the samples were put in 2-mL plastic tubes, submerged in Liquid Nitrogen Biological Container (YDS-3, −196 °C) before being stored in a freezer at −80 °C for fatty acids analysis.

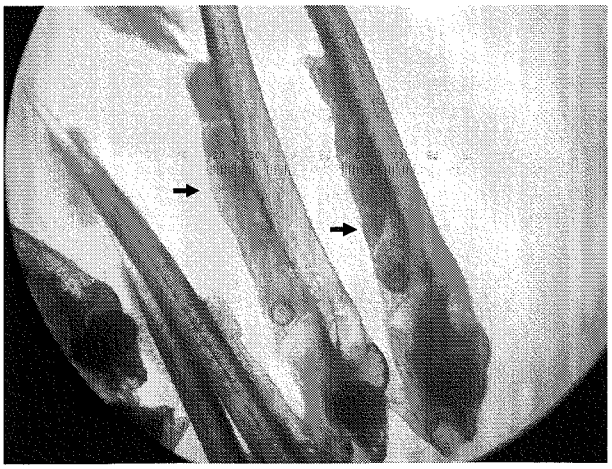

Fig. 2. Evidence of AF umbrellas in digestive tracts of cobia larvae.

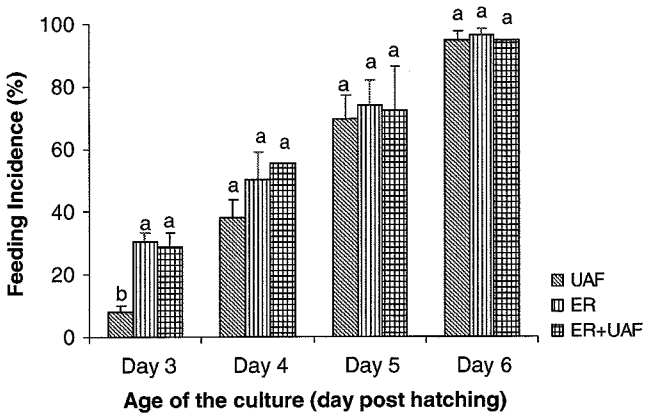

Fig. 3. Feeding incidence of cobia larvae in first feeding stage. Value (average±S.E.M) followed by different superscript letters at the same age are significantly different ($P<0.05$). ER=Enriched rotifers; ER+UAF=Enriched rotifer followed by AF umbrella; UAF=AF umbrella.

The fatty acids were analyzed according to the standard protocol described in Coutteau and Sorgeloos (1995) and were expressed in mg g^{-1} dry weight.

2.5. Evaluation of feeding, growth, survival and viability

The size of rotifer loricae (Fu et al., 1991), AF umbrella, as well as fish larval mouth opening (jaw length) at first feeding was measured under a microscope to compare and check for ingestion at first feeding of the cobia larvae. Feeding incidence was evaluated by examining 20 cobia larvae collected randomly 30 min after the last feeding daily. The sampling continued until all larvae had fed.

Larval length (using a stereo microscope at a magnification of 1.8×10; for the size bigger than 5 mm using a Mitutoyo ruler No.8355) and larval density (volumetrically counted at night time using 250 or 500-mL beakers) were randomly sampled ($n=20$) and measured at 3 dph (first feeding), 8 dph (completion of different feeding regimes) and 18 dph (start weaning period) to evaluate growth and survival in all treatments. The samples were also visually inspected under the microscope for deformities.

The quality of the cobia larvae was estimated by the Cumulative Stress Index (CSI) and Mortality Rate (MR) upon exposure of the larvae to a high salinity shock (modified from Dhert et al., 1990, 1992) at 8 dph and 18 dph. The right salinity concentration was determined in a range-finding test of 40, 50, 60, 70, 80, 90 and 100 g L^{-1} in which at least 50% of the larvae died within 1 h. Water was taken from the

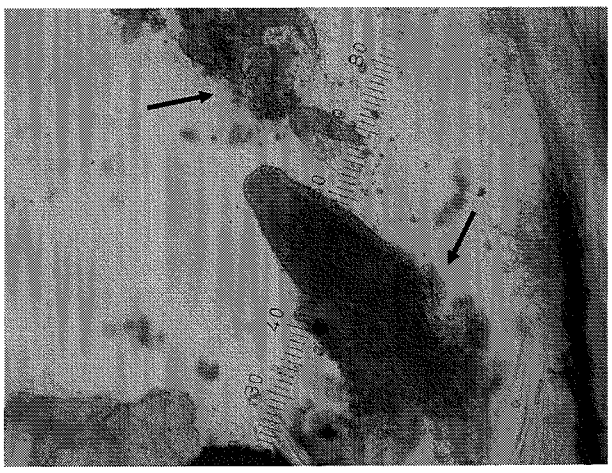

Fig. 4. Evidence of digested AF umbrella in the digestive tract of cobia larvae at day 3 post-hatching.

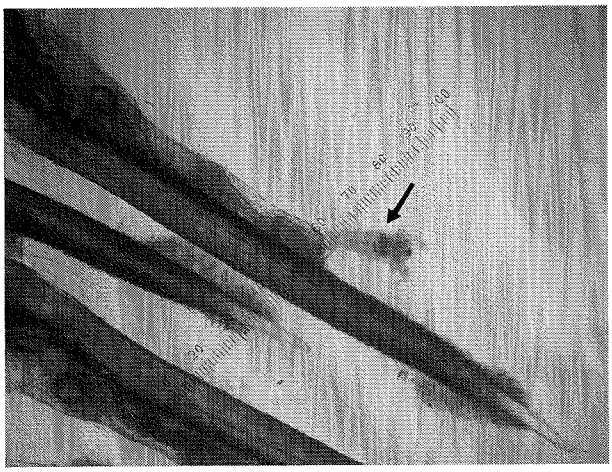

Fig. 5. Evidence of undigested nauplii leaving the digestive tract of cobia larvae at day 4 post-hatching.

rearing tank to avoid any changes in water temperature and NaCl was added to adjust the salinity to the desired concentration. For evaluation, 10 larvae were randomly sampled from each culture tank and put in a 500-mL beaker using 3 replicates for each tank. The number of dead larvae in each beaker was counted every 3 min for 60 min. The average of CSI and MR of each treatment was calculated from the replicates of each tank. The higher CSI and MR, the lower the quality of the larvae.

2.6. Statistical analysis

Feeding incidence, growth, survival and viability of cobia among the treatments were tested for significant differences ($P<0.05$) using one-way ANOVA followed by a Fisher LSD test for pair comparisons of means using SPSS version 13.0 and Microsoft Office EXCEL for Windows. The data were expressed as mean±S.E.M.

3. Results

3.1. Size suitability and digestibility of AF umbrella Artemia at first feeding of cobia larvae

At first feeding, the cobia larvae were 4.9±0.2 mm with a mouth opening of 551.0±9.7 μm. AF umbrellas were 219.4±2.0 μm in width and 378.4±3.0 μm in length, while the rotifer loricae were 100.8±3.1 μm, respectively 148.6±4.2 μm. Although AF umbrellas were bigger than rotifers, they were more uniform in size and early detection of AF umbrellas in the digestive tract (Fig. 2) confirmed their ingestion.

On the first day of exogenous feeding, the feeding incidence in the UAF treatments (0.08±0.02) was significantly ($P<0.05$) lower than in the ER (0.31±0.03) and ER+UAF (0.29±0.04) treatments. As of the next days, feeding incidence in all treatments was not significantly different ($P>0.05$) although at 4 dph, feeding incidence of UAF treatments was still lower (0.38±0.06) than in ER (0.50±0.09) and ER+UAF (0.55±0.00) treatments. As of 5 dph, about 70% of the larvae in all treatments was found with full stomachs (Fig. 3). However, in all treatments some larvae had empty stomachs till 6 dph. Digestion of AF umbrella *Artemia* was evident (Fig. 4), while some of the newly-hatched *Artemia* nauplii (developed from uneaten AF umbrella) were found passing through the digestive tract without being digested (Fig. 5).

Table 2
Highly unsaturated fatty acids (% total fatty acids) of experimental live foods

	Enriched rotifers	AF umbrella	AF nauplii	Enriched EG nauplii
20:4(n−6) (ARA)	1.62	–	–	–
20:5(n−3) (EPA)	7.97	8.78	5.22	2.51
22:6(n−3) (DHA)	16.54	–	–	6.67
(n−3) HUFA	25.91	8.86	9.18	9.18
(n−6) HUFA	1.62	–	–	–
Sum HUFA	27.53	8.86	9.18	9.18

ARA = Arachidonic acid; EPA = Eicosapentaenoic acids; DHA = Docosahexaenoic acid; HUFA = Highly Unsaturated Fatty Acids defined as fatty acids with at least 20 carbon atoms and more than two double bonds.

Table 3
Growth and survival of cobia larvae at 8 dph and 18 dph

Treatments	Standard length (mm)		Survival (%)	
	8 dph	18 dph	8 dph	18 dph
ER	6.4±0.1^{a}	11.4±0.6^{a}	75.7±11.1^{a}	16.6±3.4^{a}
ER+UAF	6.2±0.1^{ab}	11.6±0.6^{a}	68.9±12.3^{a}	12.0±1.7^{a}
UAF	6.2±0.1^{b}	11.3±0.7^{a}	60.8±11.2^{a}	14.4±2.0^{a}

Value (average±S.E.M) followed by different superscript letters within a column are significantly different ($P<0.05$). ER=Enriched rotifers; ER+UAF=Enriched rotifer followed by AF umbrella; UAF=AF umbrella.

3.2. Fatty acid profile of the live feed

The percentage of selected essential fatty acids of AF umbrella, enriched rotifers, newly-hatched (Instar I) and enriched meta-nauplii of *Artemia* are presented in Table 2. The essential fatty acids, including eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and other highly unsaturated fatty acids (HUFAs) of the enriched rotifers, reflect the lipid composition of the enrichment products. AF umbrellas, AF nauplii and enriched EG meta-nauplii were very low in DHA and arachidonic acid (ARA). AF umbrella contained mainly EPA at 8.78% of total fatty acids (Table 2) equivalent to 17.33 mg g^{-1} dry weight. The EPA content, however, decreased as umbrella developed into nauplius. Although the DHA content of enriched EG meta-nauplii was as high as 6.67%, the percentage of total HUFAs was not different from the levels found in AF nauplii. The total HUFAs content in AF umbrella was lower than that of the AF nauplii, enriched EG nauplii and much lower than that of the enriched rotifers.

3.3. Growth and survival of cobia larvae

The cobia larvae (SL in mm) of the UAF treatment were a little smaller than the ones of the ER treatments ($P<0.05$) on 8 dph, but no significant difference ($P>0.05$) was detected on 18 dph (Table 3). Larval survival decreased in all treatments from 8 dph onwards. The differences in survival were not significant ($P>0.05$).

3.4. Quality and deformity of cobia larvae

Cumulative Stress Index and Mortality Rate of cobia larvae at 8 dph exposed to 60 g L^{-1} salinity for 60 min were relatively low in ER treatments and significantly different ($P<0.05$) with the UAF and ER+UAF treatments. At 18 dph, the opposite trend was observed although Mortality Rates were not significantly different ($P>0.05$). Furthermore, no difference in larval deformities could be detected (Table 4).

Table 4
Salinity stress resistance (at 60 g L^{-1}) and deformity rates of cobia larvae at 8 dph and 18 dph

Treatments	8 dph			18 dph		
	CSI	MR	Deformity (%)	CSI	MR	Deformity (%)
ER	46.61±9.63^{a}	64.6±9.3^{a}	2.2±2.2^{a}	89.69±7.79^{a}	92.4±2.3^{a}	4.4±2.9^{a}
ER+UAF	81.42±14.33^{b}	93.9±3.8^{b}	1.1±1.1^{a}	60.28±15.73^{b}	85.3±7.5^{a}	5.6±2.9^{a}
UAF	74.54±15.76^{b}	92.0±8.0^{b}	4.4±2.0^{a}	56.82±9.53^{b}	81.2±6.3^{a}	3.3±1.9^{a}

Values (average±S.E.M) followed by different superscript letters within a column are significantly different ($P<0.05$). CSI=Cumulative Stress Index; MR=Mortality Rate; ER=Enriched rotifers; ER+UAF=Enriched rotifer followed by AF umbrella; UAF=AF umbrella.

4. Discussion

4.1. AF umbrella ingestion

In first-feeding, fish larvae prey ingestion is function of prey size (Planas and Cunha, 1999; Pena-Aguado et al., 2007; Pepin and Penney, 1997; Van der Meeren, 1991a). According to Munk (1997), the preferred prey size in cod larvae is about 5% of its larval length. For gilthead sea bream larvae, Fernandez-Diaz et al. (1994) reported that the preferred live and inert prey size was a function of mouth size showing the preference for prey in the range of 0.1–0.8 times mouth width. Although there is a positive correlation between jaw opening size and prey size, marine larval fish switch to bigger prey slower than their physical capacity to ingest larger prey (Pepin and Penney, 1997). According to Faulk et al. (2007b), cobia larvae open mouth and anus by late 2 dph with consequent differentiation of digestive organs in buccopharynx, oesophagus, stomach and intestine and are ready for first feeding by 3 dph (Faulk and Holt, 2003, 2005; Faulk et al., 2007b; Liao et al., 2001). As the jaw opening of first-feeding cobia larvae in our experiments averaged 551.0±9.7 μm, larvae were able to ingest the AF umbrella *Artemia* of 378.4±3.0 μm. However, the low feeding incidence in the UAF treatment recorded at initial exogenous feeding might have several reasons: as the umbrellas do not move, they might be less attractive and even when delivering the umbrellas via a dripping device, they might not create a homogeneous distribution of AF umbrella as with live rotifers. The increase of feeding incidence in all treatments (including the ER treatments) in the following days might be related to eye development resulting in more effective prey detection and consequent ingestion.

4.2. Digestion of AF umbrella

It is well documented that at start feeding, the digestive system of fish larvae is not fully functional yet (Kolkovski, 2001). Microalgae are actively ingested at early larval stages e.g. cod *Gadus morhua* (Van der Meeren, 1991b) and appear, in some marine species to function as a trigger for further maturation of the digestive system (Reitan et al., 1998, 1997). It is believed that supplementation of digestive enzymes from live prey is important to further support digestion in early developmental stages of most marine fish larvae (Kolkovski, 2001; Kolkovski et al., 1997; Munilla-Moran et al., 1990). As documented by Faulk et al. (2007b), the digestive system in cobia larvae is functional as of mouth opening, i.e. enzymes and zymogen granules are present in the pancreas. Moreover, the majority of morphological changes observed in the gastrointestinal tract occurred over the first 1–4 dph (3.6–4.4 mm) and the enzymatic activity for trypsin, lipase and especially amylase increased by 3–4 dph to return to relatively low levels by 8–12 dph (5.7–8.1 mm), at which point a general increase was observed for all enzymes examined (Faulk et al., 2007b). As AF umbrellas are actively metabolizing embryos, they might already contain free amino acids and fatty acids resulting from protein and lipid hydrolysis during embryonic development (Garcia-Ortega et al., 1998).

In our experiment, the oil globule of cobia larvae was depleted by 8 dph at 28.0±0.1 °C, similar to the description of Faulk et al. (2007b) that the yolk sac was completely absorbed by 3–4 dph, followed by a rapid decrease in oil globule size, disappearing entirely by 9–10 dph at a rearing water temperature of 25.9±0.7 °C. If suitable food is not ingested in time, 8–9 dph is a critical period for survival. The high survival in the UAF-fed treatments at 8 dph revealed that at least 60% of the cobia larvae could rely on AF umbrella for this rearing period.

4.3. Nutritional value of AF umbrella

It is well documented that *Artemia* nauplii are a suitable prey for the larvae of many fish species (Sorgeloos et al., 2001). Although cobia larvae have a high DHA requirement (Faulk and Holt, 2005), DHA-deficient umbrella *Artemia* appear to be a suitable starter diet in comparison to DHA-enriched rotifers. Fast-growing cobia larvae have high energetic requirements and the amount of calories ingested per prey catching effort could be critical, especially as the larvae are growing, i.e. a significantly smaller net energy gain per gram DW food uptake (calories ingested minus calories spent to catch more prey) is expected when feeding too long on a diet of small rotifers. This may not be critical at start feeding, but it certainly becomes critical within the following days as was documented by N. King (in Fish Farming International, January 2006) and by Holt et al. (2007): each day of advancing *Artemia* feeding (by switching to a strain producing smaller nauplii) resulted in improved survival and growth.

The higher stress sensitivity at 8 dph in the umbrella-fed fish might result from the dietary DHA deficiency. According to Rainuzzo et al. (1997) the inappropriate HUFAs ratios, such as DHA:EPA ratio, may create an imbalance of the structural composition of the phospholipids which could affect the normal growth and the quality of the larvae. The DHA level was very high in yolk sac larvae (Faulk and Holt, 2003; Holt et al., 2007) and there was a positive correlation with the DHA level in the larval tissue at 16 dph and in live prey, except for EPA and ARA (Faulk and Holt, 2005). A correlation between low stress tolerance and larvae fed diets low in DHA has been documented in many marine fish species (Brinkmeyer and Holt, 1998; Kanazawa, 1997; Om et al., 2001). Although the DHA and the total (n−3)HUFAs levels were higher in enriched rotifers as compared to AF umbrella, no significant difference in growth and survival was detected in the larvae fed those diets on 18 dph. This confirms the finding of Faulk and Holt (2005) and Benetti et al. (2007) that in terms of prey enrichment, rotifers may be less important than *Artemia* for cobia larvae due to the short rotifer-feeding period. The requirement for essential fatty acids of cobia larvae during the rotifer and *Artemia* feeding period is still not resolved.

It is also important to remind that growth and survival of marine larval fish not only depends on exogenous nutrition and digestive and metabolic enzyme capacity of newly-hatched larvae (Lamarre et al., 2004), but also on egg quality and consequently broodstock nutrition (Navas et al., 1997; Watanabe and Vassallo-Agius, 2003). In this experiment, cobia larvae were obtained from broodstock fed a diet with a high level of HUFAs, which is reflected in high DHA levels in the newly-hatched larvae.

In summary, it is possible for cobia larvae to ingest and digest AF umbrella since first feeding. Replacing enriched rotifers by AF umbrella-stage *Artemia* as starter food for cobia larvae has very little effect on growth by 8 dph and appears to have no significant negative effect on larval quality, growth or on survival by 18 dph. The successful use of AF umbrella opens a possibility to simplify the rearing protocol for cobia larvae, especially through a reduction of labour costs for live food production. Researches on the use of AF umbrella including production and improvement of the availability in larval rearing tanks should be addressed. It is also important to consider research on co-feeding of AF umbrella with formulated feeds to adjust the nutritional balance and thus compensate for the suboptimal nutritional value of AF umbrella for early larval growth and survival in cobia.

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