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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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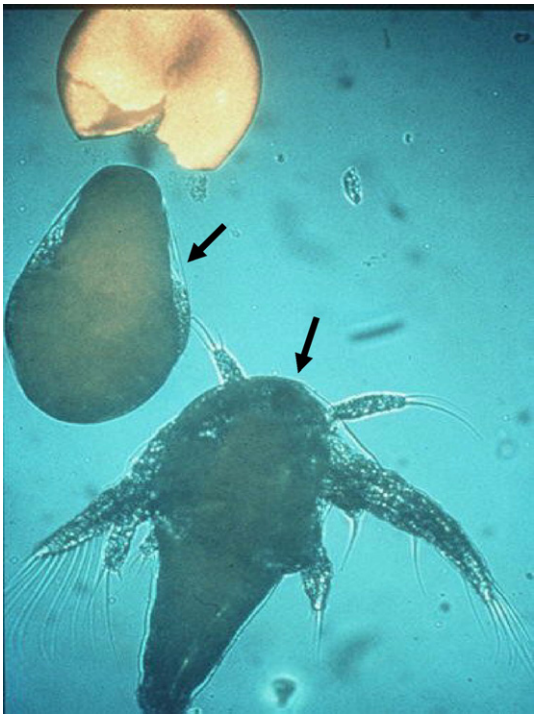


Fig. 1. Development of an *Artemia* cyst into umbrella-stage and instar I nauplius stage (Lavens and Sorgeloos, 1996).

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).

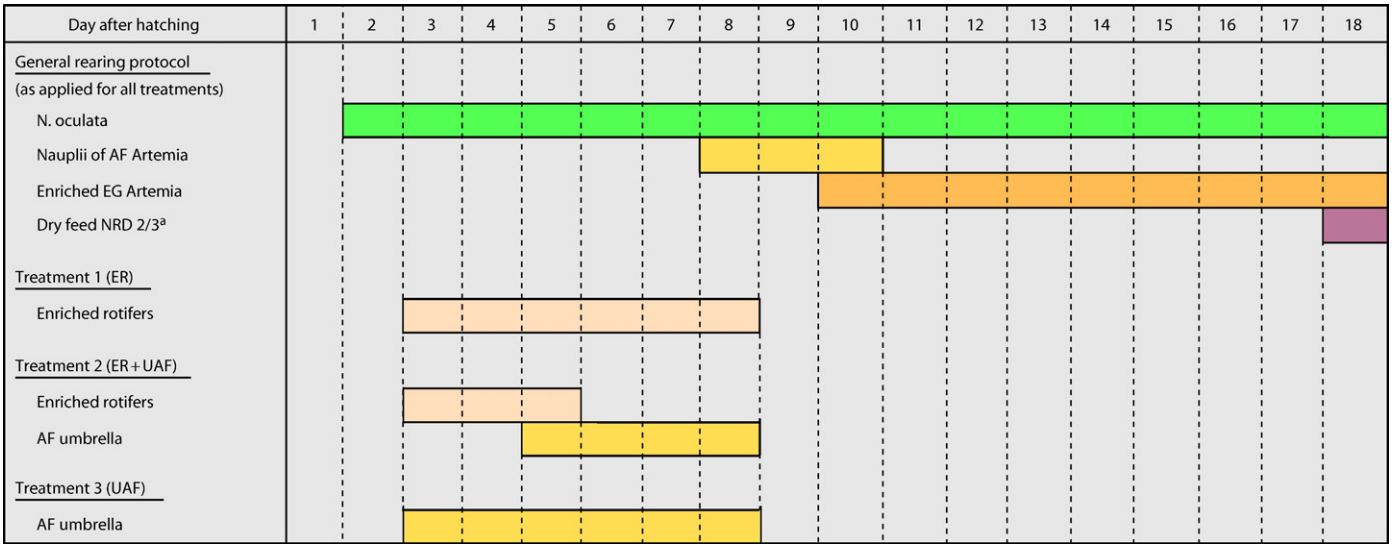
2.2. Live food preparation

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



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