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Use of the copepod *Acartia tonsa* as the first live food for larvae of the fat snook *Centropomus parallelus*

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ABSTRACT

High-quality live food is essential for reducing the frequent high mortality of newly hatched fat snook (*Centropomus parallelus*) larvae in hatcheries. Copepods, a rich nutrition source, cultivated with the microalgae *Chaetoceros muelleri* and *Isochrysis galbana*, were evaluated as food for 0–14-day-old larvae. Two experiments were performed using nine 50-L tanks stocked with 2500 embryonated fat snook eggs. Three different dietary (treatments) were tested in triplicate: Experiment 1, Treatment 1 (Control), using rotifers *Brachionus rotundiformis* (20 mL^{-1}); Treatment 2 (Copepod), larvae were fed with *Acartia tonsa* (nauplii and copepodits, 0.1 mL^{-1}); and Treatment 3 (Mixed), larvae were fed with *A. tonsa* (0.05 mL^{-1}) and rotifers (10 mL^{-1}). In Experiment 2 to increase the density of live food, food organisms and the phytoplankton were introduced into the experimental tanks with the embryonated eggs and were stocked: Treatment 1, 20 rotifers mL^{-1}; Treatment 2, 0.5 copepods mL^{-1}; and Treatment 3, 10 rotifers mL^{-1} and 0.25 copepods mL^{-1}. In Experiment 1, the Mixed Treatment increased significantly the survival rate (16.0% ± 7.5%) and mean larval weight (0.84 ± 0.05 mg) in relation to the other treatments. In Experiment 2, we observed significant improvements in larval notochord flexion in the Copepod and Mixed Treatment. The essential fatty acid profile of fat snook eggs had a DHA:EPA:ARA ratio of 11.4:2.4:1.0 while larvae in the Mixed and Copepods Treatments had ratios of 2.5:1.9:1.0 and 5.5:1.9:1.0, respectively. We conclude that the survival, development and the relationship between the major fatty acids were improved in treatments with copepods.

1. Introduction

Larval rearing is still the limiting step for the development of industrial marine aquaculture of fish of the genus *Centropomus* and many other species, including the groupers *Epinephelus septemfasciatus* and *E. marginatus* (Liao et al., 2001; Russo et al., 2009; Sakakura et al., 2007), the Asian sea bass *Lates calcarifer* (Rajkumar and Vasagam, 2006), the yellowtail *Seriola lalandi* (Chen et al., 2006) and the mutton snapper *Lutjanus analis* (Benetti et al., 2002; Watanabe et al., 1998).

Marine fish hatcheries generally suffer high mortality due to the fragility of the larvae in the early stages of their development. The larvae undergo drastic morphophysiological changes (Alvarez-Lajonchère et al., 2002a; Chen et al., 2006; Yúfera and Darias, 2007) to adapt to the habitat, facilitate the capture of prey and assimilate nutrients, which are the basic prerequisites for growth and survival. Therefore demand of nutritional quality makes the lipids to be considered of great importance during this phase (Izquierdo et al., 2000).

The fat snook (*Centropomus parallelus*), a species of great economic and social importance in the warm waters of the Atlantic coast of the Americas (Rivas, 1986), has been studied for use in aquaculture (Cerqueira and Tsuzuki, 2009). High mortality rates after the start of exogenous feeding (day 3), have been observed since the earliest hatchery trials (Cerqueira, 1991; Cerqueira and Brugger, 2001; Cerqueira et al., 1995; Seiffert et al., 2001), resulting in <10% survival by the end of 14 days, when the larvae begin notochord flexion. After this phase, mortality tends to decrease significantly.

The copepods are a major natural food source for marine fish larvae and have more nutritional value than rotifers and *Artemia* sp., normally used in intensive marine fish hatcheries (Stottrup and Norsker, 1997), since the production techniques have already been mastered. Copepods are a rich source of essential nutrients, especially highly unsaturated fatty acids (HUFAs) such as docosahexaenoic acid

Abbreviations: dah, days after hatch.

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(DHA, 22:6 n−3) and eicosapentaenoic acid (EPA, 20:5 n−3) (Stottrup et al., 1999). Another advantage of the copepod is its size, which is ideal for the small pelagic larvae of marine fish (Rajkumar and Vasagam, 2006; Schipp et al., 1999). Calanoida copepods of the genus *Acartia* have been grown experimentally in other studies for use as a first live food (Marte, 2003; Rajkumar and Vasagam, 2006; Schipp et al., 1999; Stottrup, 2006; Yanes-Roca and Main, 2012).

Given the advantageous qualities of copepods and the necessity to increase initial survival during the cultivation of fat snook larvae, the main objective of this study was to compare the copepod *Acartia tonsa* with the rotifer as a first live food, based on the production performance of the larvae and the fatty acid profiles of both foods.

2. Materials and methods

2.1. Algal culture

Microalgae were produced according to the method described by Lourenço (2006) and were used once they reached a density of 70×10^4 cells mL^{-1} for *Chaetoceros muelleri* and *Isochrysis galbana*, and 100×10^4 mL^{-1} for *Nannochloropsis oculata*.

2.2. Copepod culture

Wild copepods were collected in Lagoa da Conceição (Florianopolis, SC) and ponds close to the laboratory. The collection apparatus comprised of 20-L bucket with two 200-micron screen windows, coupled with an external airlift water system. Every morning the collected samples were sieved through a 650-micron mesh screen, to remove large material, and then through a 200-micron mesh screen, to retail adult copepods (>90% *A. tonsa*). These individuals were stocked in a 500-L cylinder-conical tank containing *C. muelleri* and *I. galbana* at 30×10^4 cells mL^{-1} and 60×10^4 cells mL^{-1}, respectively, salinity of 35 g L^{-1} and temperature of 26 °C. After acclimation in the laboratory, the copepods *A. tonsa* adults were transferred to a 250-L cylinder-conical tank, where nauplii were produced according to the technique developed by Bersano (2003). This production tank had an egg nauplii collector with a 45-micron mesh screen. The nauplii were reared under the same feeding conditions, temperature and salinity, requiring 8 days to become adults. These cultures were always started in small containers (3 L), and the volume was gradually increased until reaching a volume of 40-L tank for the production of adults. Nauplii and copepodites were used to feed snook larvae at the beginning of the experiments, but adults were also included in the diet of older larvae.

2.3. Rotifer culture

Culture containers (2 L) of the rotifer *B. rotundiformis* were maintained at a controlled water temperature of 28 °C, with a salinity of 25 g L^{-1}, and were fed with *N. oculata* at a density of 100×10^4 cells mL^{-1} for seven days. The upscaling of the rotifers was carried out in 40-L and subsequently in 500-L cylinder-conical tanks with constant aeration, where they were fed with dry yeast (*Saccharomyces cerevisiae*; 1 g/10^6 rotifers), microalgae *N. oculata* (100×10^4 cells/rotifer^{-1}) and Culture Selco (INVE ®, Belgium; 0.5 g/10^6 rotifers, day 3 only). Once the rotifers have reached a density of 500 individuals mL^{-1} they were used in the larval feeding experiments.

2.4. Growth trials

The study was conducted at the Laboratorio de Piscicultura Marinha (LAPMAR) of the Universidade Federal de Santa Catarina (27° 37″ S and 48° 27″ W, Florianopolis, Santa Catarina State, Brazil) and was part of a doctoral thesis which developed experiments with larvae and post-larvae of fat snook from 0 to 14 dah, 15 to 27 dah and 31 to 45 dah and juveniles of 50 to 140 dah.

Snook larvae for the experiments were obtained by means of induced spawning, according to Ferraz et al. (2002). Fertilization was natural and the eggs were collected from a 200-L incubator, with constant aeration and temperature (26 °C). The larval tanks were introduced before the onset of hatching. Two experiments were conducted in nine 50-L cylindrical tanks with fat snook larvae and stocking density of 50 embryonated eggs L^{-1}, in order to test different feeding regimes. The larvae were cultivated until fourteen day-old. Daily water change begun at 10% of the tank volume on the fifth day and was gradually increased to 25% on the ninth day, which rate was maintained until the end of the experiment. Three treatments were performed with 3 replicates each.

2.4.1. Experiment 1

In Treatment 1 (Control) larvae were fed with the rotifers *B. rotundiformis* (3–20 mL^{-1}); in Treatment 2 (Copepod), larvae were fed with *A. tonsa* nauplii and copepodits at 0.1 mL^{-1}; in Treatment 3 (Mixed), larvae were fed with nauplii and copepodits of *A, tonsa* at 0.05 mL^{-1} and rotifers at 10 mL^{-1}. *N. oculata* were added daily at 100×10^4 cells mL^{-1} in all treatments. The density of live food in each tank was checked every morning before additional food was added. Daily consumption was virtually 100% throughout the experiment. The following water quality variables were monitored daily and maintained as indicated: temperature (26.0 ± 1.0 °C), salinity (35.0 ± 1.0 g L^{-1}) and dissolved oxygen (5.4 ± 0.4 mg L^{-1}). The larval survival and growth (in total length and weight) were quantified and compared between treatments.

2.4.2. Experiment 2

In order to increase the density of live food, food organisms and the phytoplankton with whom they are fed were introduced into the experimental tanks with the embryonated eggs (3 days before the end of the lecithotrophic phase). The environmental conditions were favorable for the cultivation of zooplankton (poor aeration and temperature around 26 °C). The control treatment tank was stocked with rotifers (20 mL^{-1}) and *N. oculata* (100×10^4 mL^{-1}), according to the protocol of Alvarez-Lajonchère et al. (2002a). For Treatment 2 the tanks were stocked with copepods (nauplii, copepodits, and adults, 0.5 mL^{-1}), *C. muelleri* (30×10^4 mL^{-1}) and *I. galbana* (60×10^4 mL^{-1}). The Mixed Treatment tanks were stocked with rotifers (10 mL^{-1}), copepods (0.25 mL^{-1}), and the 3 microalgae at the same densities described above.

The tanks had individual water supplies and drainage (with a 120-mm screen at the outlet), although the water was not replaced and only microalgae were added for the first 4 days. On later days, the water was replaced as in Experiment 1.

The density of live food was monitored each morning, before any additional food was added. After this daily count, live food was added to maintain the stipulated minimum densities for each treatment.

Water quality variables such as temperature (27.9 ± 0.9 °C), salinity (35.0 ± 1.0 g L^{-1}) and dissolved oxygen (5.6 ± 0.6 mg L^{-1}) were monitored daily. The survival and growth (in total length and wet weight) of the larvae were quantified. The condition factor (K) was calculated by: $(W/L^3)\times100$, where W = weight (mg) and L = length (mm). Larval development was evaluated by the presence of a functional gas bladder and the beginning of notochord flexion by using an optical microscopy during the final biometrics analysis.

2.5. Biochemical analysis

Samples of rotifers, copepods, eggs and 14 day-old fat snook larvae were collected from the different treatment tanks and stored in sealed glass tubes at −80 °C. Analyses were performed at the Laboratory of Fatty Oils at Embrapa Agroindustria de Alimentos, RJ. Each sample