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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

was homogenized with a sonicator (Branson Sonifier 250 set to output control, micro tip unit 7, duty cycle 30) for 2 min.

The lipids were extracted according to the method of Bligh and Dyer (1959) using the solvents chloroform, methanol, and water at a ratio of 1:2:0.8 for the initial extraction and final proportions of 2:2:1.8 to obtain a biphasic solution. The triglyceride triundecanoin (C11) was added as an internal standard.

The fatty acid methyl esters (FAME) were prepared according to the method of Hartman and Lago (1973). Gas chromatography was performed using an Agilent 6890 chromatograph fitted with a cyanopropyl siloxane capillary column (60 m × 0.32 mm × 0.25 mm). Results were expressed as weight percentage (area normalization) and as by-weight of the dry material. The components were identified by comparing their retention times against those of manufacturer standards (Nu-Chek Prep® Inc., Elysian, USA) and the standard marine-source PUFA and PUFA 3 Menhaden oil (Supelco®, Bellefonte, USA). Quantification of the proportions of identified fatty acids was performed by comparing the areas under the curves after normalization in relation to the internal standard. The standard deviation could not be calculated due to reduced amount of material samples, although they matched the fatty acids of juvenile fat snook analyzed in the referred doctoral thesis.

2.6. Statistical analysis

Analysis of variance was performed on evaluated larval parameters (survival, weight, length, condition factor, presence of gas bladder, and flexion of the notochord), after confirmation of the homoscedasticity of variances and normality of data distribution. The data for survival rate, the presence of gas bladder and the beginning of notochord flexion were subjected to the arcsine transformation prior for testing. The mean and standard deviation are given for each variable. Where significant differences between treatments ($\alpha = 0.05$) were detected. The Tukey HSD test was applied to separate means. All tests were performed using STATISTICA software version 6.0.

3. Results

In Experiment 1 the Mixed Treatment showed a significantly higher survival (16.0% ± 7.5%) and mean weight (0.84 ± 0.05 mg) compared to the Control Treatment (5.0 ± 5.2% and 0.71 ± 0.02 mg, respectively), as shown in Fig. 1a and b. Mortality was 100% in Copepod Treatment, probably due to a shortage of food (mean 0.04 copepods mL^{-1}) as the other growing conditions were the same of the other treatments.

In Experiment 2 newly hatched nauplii and copepodits of *A. tonsa* were observed in the counted collected samples of remaining live food, indicating that adult copepods were reproducing in the experimental tanks. In the Copepod Treatment the average density was 4 mL^{-1}, but the density varied greatly and reached 0 on the sixth and tenth days. For the Mixed Treatment, the copepod density average was 2 mL^{-1} but reached 0 on days 4 and 8 and the last 3 days and the rotifer density increased up until to the sixth day and then gradually declined. In the Control Treatment the density of rotifers remained stable throughout the experiment.

There was no significant difference in survival between the treatments, with a minimum of 3.5% in the Copepod Treatment and a maximum of 11.3% in the Mixed Treatment (Table 1). Likewise, no significant difference in length or formation of the gas bladder was observed.

The Mixed Treatment was significantly higher than the Rotifer Treatment in larval weight, but in Copepod Treatment there was no significant difference in relation to the others (Fig. 2a). In the other indicators, treatments that included copepods (Copepod and Mixed) had significantly higher values than the treatment without copepods (Rotifer) for the condition factor and the development of the larvae (flexion of the notochord). The Mixed Treatment gave the best values for the developmental indicators (Fig. 2a and b).

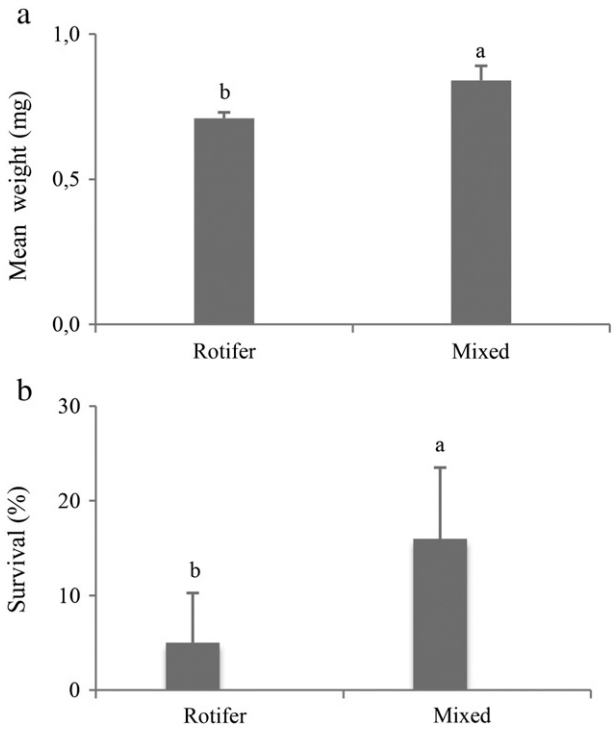

Fig. 1. Mean weight (a) and survival (b) of 14-day-old fat snook (*Centropomus parallelus*) larvae in 2 treatments in Experiment 1: Rotifer and Mixed (Rotifer + Copepod).

The fatty acid compositions of the zooplankton, the fat snook eggs and larvae were divided into saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids, presented in Table 2.

The cultivated copepods had the highest amounts of SFA (498 mg g^{-1}), which exceeded even those of the wild copepods (351 mg g^{-1}). The magnitude of these values occurred due to the large amounts of palmitic acid (16:0), myristic acid (14:0) and stearic acid (18:0), presented in the cultivated copepods. MUFA fatty acids were also more abundant in cultured copepods (411 mg g^{-1}) than in wild copepods (161 mg g^{-1}) due to higher amounts of oleic (18:1 n − 9) and palmitoleic (16:1 n − 7) acids. The amounts of PUFA were higher in wild copepods (360 mg g^{-1}) than in cultured copepods (67 mg g^{-1}). Large amounts of DHA and EPA (170 and 96 mg g^{-1}, respectively) were found in wild copepods. The cultured copepods were comparatively poor sources of these fatty acids. Arachdonic acid (ARA) was not detected in cultured copepods and was less than 1% in wild copepods with an EPA/ARA ratio of about 10:1.

Rotifers contained high levels of oleic and palmitoleic acids and thus had 455 mg g^{-1} of MUFA. The total PUFA was only 161 mg g^{-1}, with large amounts of EPA and ARA and only a tiny amount of DHA (3.4 mg g^{-1}). They also showed accumulation of trans fats (96 mg g^{-1}), although no similar accumulation was observed in rotifer-fed larvae.

Table 1
Mean (± standard deviation) of total length, condition factor, survival rate and functional gas bladder formation of 14-day-old fat snook (*Centropomus parallelus*) larvae in Experiment 2.

	Treatments		
	Rotifer	Mixed	Copepods
Total length (mm)	3.75 ± 0.49^{a}	3.86 ± 0.22^{a}	3.56 ± 0.36^{a}
Condition factor (K)	1.02 ± 0.25^{b}	2.09 ± 0.27^{a}	1.82 ± 0.22^{a}
Survival (%)	4.7 ± 1.2^{a}	11.3 ± 4.1^{a}	3.5 ± 5.1^{a}
Functional gas bladder (%)	90.0 ± 14.6^{a}	100.0 ± 0.0^{a}	92.8 ± 8.6^{a}

Values are expressed as mean ± SD.
Values in the same row with different superscripts are significantly different ($P<0.05$).

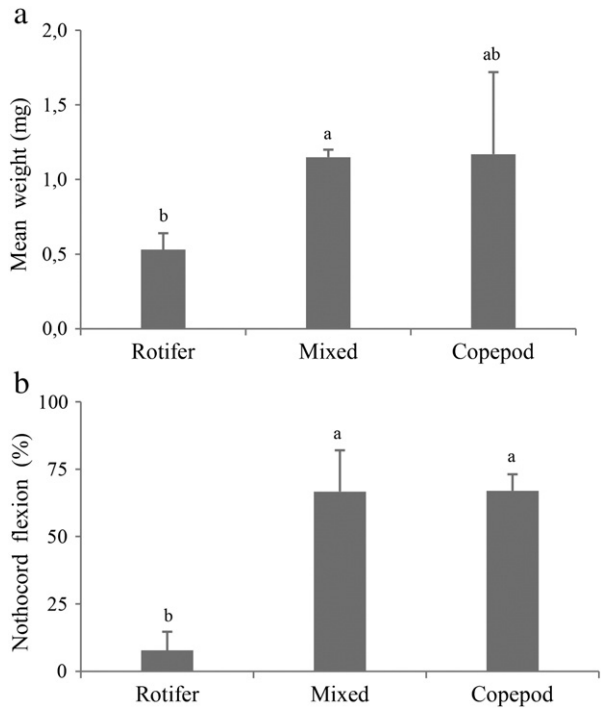

Fig. 2. Mean weight (a) and percentages of 14-day-old fat snook (*Centropomus parallelus*) larvae with nothocord flexion (b) in 3 treatments in Experiment 2: Rotifer, Mixed (Rotifer + Copepod), and Copepod.

The larvae from all treatment groups contained high amounts of SFA (>400 mg g^{-1}), depending on the amounts of palmitic, stearic and myristic acids, and low amounts of MUFA (180–210 mg g^{-1}), with rotifer-fed larvae having the highest level. The larvae of the Copepod and Mixed Treatments had higher DHA contents (170 and 130 mg g^{-1}, respectively) than larvae of Control Treatment (rotifers, 54 mg g^{-1}). The EPA and ARA contents were the highest in the larvae from the Mixed Treatment.

Analysis of fat snook eggs showed SFA values about 40% smaller than those of the larvae with the largest amount of PUFA of any sample, due to high levels of DHA (227 mg g^{-1}) and linoleic acid (92 mg g^{-1}). The MUFA levels were similar in eggs and larvae, with a slightly greater concentration in the eggs.

4. Discussion

The fragility and drastic morphophysiological changes undergone by newly hatched fat snook larvae frequently result in high mortality during their rearing, which was indeed observed in this study from the ninth day after hatching. The final mean survival rates from both experiments ranged from 3.6% to 16%, and there were no significant differences between treatments in the second experiment. However, this study showed best survival rate than the study of Yanes-Roca and Main (2012), that had significant highest mean survival (1.4%) in feeding treatments which combinated copepods *A. tonsa* (75%) and rotifers (25%) for common snook larvae with 14-day-old, respectively.

High mortality at this stage was described by Cerqueira et al. (1995) and Alvarez-Lajonchère et al. (2002b), who reported the early post-hatching days to be the critical for hatchery rearing of *C. parallelus* species. Using early-stage fat snook larvae, Seiffert et al. (2001) obtained survival close to 1%, while Cerqueira and Brugger (2001) performed 4 larval experiments, of which 2 had average survival rates of less than 1% and the others ranged from 0.4% to 16.0%. The best results for survival of newly hatched fat snook larvae, averaging between 29.4% and 38.8% were obtained by Temple et al. (2004). The same trend has been observed in hatcheries of other marine fish species. Sugama et al. (2003), studying the larval grouper (*Cromileptes altivelis*), obtained survival rates of 2.7% to 5.1% and observed that high mortality occurred from the second to the fifth day post-hatching. Survival to 21 days was 3.8% to 16.9% for cultured larval snapper *Pagrus auratus* (Fielder et al., 2005). According Yúfera and Darias (2007), the beginning of exogenous feeding is a crucial moment in the lives of larvae, because starvation or ingestion of inappropriate food after exhaustion of the vitelline reserves greatly reduces survival. At this stage, physiological and morphological development is essential for ensuring autonomous swimming and digestibility of the ingested nutrients. As also reported by Yanes-Roca and Main (2012), this first feeding phase is critical for common snook larval survival and the synchronization between exhaustion of endogenous reserves and first feeding must occur.

Table 2
Fatty acid composition and total lipids of zooplankton, eggs and 14-day-old fat snook (*Centropomus parallelus*) larvae fed with different live foods in Experimental 2 (mg g^{-1} dw).

	Cultivated copepods	Wild copepods	Rotifers	Fat snook eggs	Larvae-fed rotifers	Larvae-fed copepods	Larvae-fed mixed
Fatty acids							
C14:0	96.3	*57.3*	*20.5*	26.7	41.6	30.5	24.6
C16:0	300.6	207.2	125.2	190.0	297.4	297.3	261.5
C18:0	86.2	62.2	*56.5*	41.2	107.8	125.9	95.1
Σ SFA[a]	*497.5*	*351.2*	234.1	267.1	*465.8*	471.2	406.2
C16:1(n−7)	123.1	47.5	162.6	*52.8*	*55.7*	47.2	*78.5*
C18:1(n−9)	*275.7*	101.0	247.3	168.6	151.2	103.7	92.8
Σ MUFA[b]	410.6	161.0	*454.5*	239.2	206.9	190.1	179.6
C18:2(n−6)	40.6	43.6	30.8	92.4	62.6	42.3	44.0
C20:4(n−6) ARA	ND	9.2	24.0	19.9	33.3	30.7	*51.7*
C18:3(n−3)	ND	33.9	8.2	21.2	ND	ND	4.9
C20:5(n−3) EPA	8.5	95.8	62.5	46.8	41.9	56.9	95.9
C22:5(n−3)	ND	6.9	31.8	29.3	37.7	ND	42.9
C22:6(n−3) DHA	14.5	170.3	3.4	226.6	54.0	168.2	127.4
Σ PUFA[c]	63.6	359.7	160.7	436.2	229.5	298.1	366.8
Σ Trans[d]	ND	65.9	95.7	20.3	19.1	ND	7.4
DHA/EPA	1.7	1.8	0.05	4.8	1.3	3.0	1.3
EPA/ARA	–	10.4	2.6	2.4	1.3	1.9	1.9
Total lipid	24.2	13.4	3.6	26.1	21.4	19.5	24.5

[a]Includes 12:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 22:0, 24:0. [b]Includes 14:1, 16:1 (n−7), 17:1, 18:1 (n−9), 20:1 (n−9), 22:1, 24:1. [c]Includes 18:2(n−6), 20:4(n−6), 18:3(n−3), 20:5(n−3), 22:5(n−3), 22:6(n−3). [d]Includes 14:1 trans; 16:1 trans; 18:1 trans; 18:2 trans; 18:2 trans trans. ND — not detected; values in each column represent 2 or 3 replicates/analysis; the unidentified peaks were not considered.

The formation of the gas bladder allows vertical displacement in the water column while flexion of the notochord is a prerequisite for the formation of the caudal fin, which is important for horizontal swimming. The development of the digestive system allows efficient digestion and nutrient absorption.

Our observed results for total length are within the range for 14-day-old C. *parallelus* larvae described by Alvarez-Lajonchère et al. (2002a) — 3.0 to 5.3 mm. However, they are smaller than those reported by Araújo et al. (2000) and Temple et al. (2004) — 4.5–4.8 mm —, but larger than those described by Seiffert et al. (2001) — 3.1–3.4 mm. We obtained a high percentage for development of the gas bladder, (90 to 100%), higher than that found by Araújo et al. (2000) — 84.1%–87.5% —, Cerqueira and Brugger (2001) — 36.8%–100% — or Seiffert et al. (2001) — 11.9%–34.7%.

In all previous studies with C. *parallelus* larvae, the rotifer was the only live food used initially. Therefore, the larval development results obtained in this study were within the expected ranges for this species. For using of copepods as first food, the main objective of this study, larvae from the Mixed and Copepod Treatments showed better performance than the Control Treatment (Rotifer). In Experiment 1, the Mixed Treatment had significantly higher survival and weight than the Control Treatment (Fig. 1a and b). While there was no significant difference in survival in Experiment 2, the Copepod and Mixed Treatments of that experiment had significantly greater percentages of larvae with flexion of the notochord, an important morphological indicator of age-appropriate development (Fig. 2b) and the condition factor was also significantly higher for these treatments than for the Rotifer Treatment (Table 1). The Condition factor is directly related to the physiological state of the larvae, presumably because individuals with greater mass for a given length are in better physiological condition (Lima-Junior and Goitein, 2006). In addition, the Mixed Treatment was significantly higher in larval weight than the Rotifer Treatment.

These results can be explained by the advantages of copepods above rotifers as an option for live food, despite the small amount of copepods offered to the larvae in this study. A good mix of microalgae was used, which was an excellent HUFA source for the copepods, and therefore also for the fat snook larvae. Moreover, according to Schipp et al. (1999) and Schipp (2006), copepods have a higher proportion of polar or structural lipids, which are more biologically available to fish larvae than triacylglycerols. The nauplii are small in size (<100 μm), easily digested and are still a good source of antioxidants, astaxanthin and vitamins C and E. Another important advantage of copepods is their jerking (i.e. zigzag) swimming motion, providing a visual stimulus for the larvae of pelagic fish, which are recognized as visual predators.

In studies on dusky grouper (*E. marginatus*), hatching in a mesocosm system (with native zooplankton, rotifers and *Artemia* sp.), Russo et al. (2009) found that larvae with a length of 2.0 to 12.0 mm and mouth opening from 0.25 and 0.70 mm preferentially selected copepod nauplii as food. Knuckey et al. (2005) also observed that *E. coioides* fed with a mixture of rotifers and copepods preferred newly hatched copepod nauplii. Alvarez-Lajonchère et al. (2002a) found that fat snook larvae have a mean length of 2.8 mm and mouth opening of 0.22 mm at the beginning of exogenous feeding; it seems that this selectivity for copepods was also present in our study, due to better performances obtained in treatments including copepods (Mixed and Copepod).

Another indicator of selectivity of larvae by copepods was the population growth of rotifers at the beginning of Experiment 2 in the Mixed Treatment's tanks. And there was availability of copepods observed in the daily morning count of the Copepod Treatment (average 0.45 copepod·mL^{-1}).

The copepod *Centropage typicus* was used for initial feeding of clownfish (*Amphiprion clarkii*) larvae at a density of 5 nauplii mL^{-1} in the first 7 days and 3 copepodits mL^{-1} in the 4 subsequent days and produced higher growth and survival than feeding with rotifer or *Artemia* spp., despite the greater supply of the latter (10 rotifers mL^{-1} and 6*Artemia* sp.mL^{-1}) (Olivotto et al., 2008). In the study of newly hatched groupers in a mesocosm system, feeding densities of 0.01–0.1 copepods mL^{-1} and 0.2–1 rotifer mL^{-1} resulted in a mean survival of 10% after 35 days of culture (Russo et al., 2009). *E. coioides* larvae had higher survival and growth when the rotifer diet was supplemented with only 0.1 copepod nauplii mL^{-1} (Knuckey et al., 2005). In this study, the Mixed Treatment larvae in Experiment 2 had the highest rates of morphological and physiological development, with 100% gas bladder inflation, 66.7% initiation of notochord flexion and higher condition factor. The Rotifer Treatment larvae showed a high percentage of gas bladder formation (90.0%), but only 7.8% of the larvae had initiated notochord flexion and weight and the condition factor was significantly smaller.

Regarding the fatty acid content of the diets used in this study, it is known that the PUFA, ARA, DHA and EPA are essential for marine fish larvae (Sargent et al., 1999a,1999b), as larvae have limited or no ability to convert linolenic acid (LNA, 18:3 n − 3) into EPA and DHA or linoleic acid (LA, 18:2 n − 6) into ARA. DHA, EPA and ARA are key components of cell membrane phospholipids and influence ion exchange and transport (endocytosis and exocytosis), and activate membrane receptor sites and enzymes, among other activities (Ibeas et al., 1997).

The ideal diet for marine fish larvae should reflect the content of the yolk sac, which is nutritionally very similar to wild copepods (Sargent et al., 1999b). As in-depth studies don't exist yet on the lipid composition and content of the yolk sac of fat snook larvae, we used the fatty acid profile of eggs obtained by artificial breeding. The eggs contained about 240 mg g^{-1} of MUFA, and around 440 mg g^{-1} of PUFA, due to the high DHA content (≅230 mg g^{-1}), giving a DHA:EPA:ARA ratio of 11.4:2.4:1.0, a DHA:EPA ratio of about 5.0:1.0 and an EPA:ARA ratio of approximately 2.5:1.0. The predominant fatty acids were the same as those of unfertilized wild snook eggs (Yanes-Roca et al., 2009), but the quantities and relative proportions varied (the DHA:EPA:ARA ratio of wild unfertilized eggs was 3.7:0.7:1.0). Few studies on fish eggs are available, but the concentration found in this study was much higher than that reported for sturgeon eggs, which had DHA levels ranging from 48 to 107 mg g^{-1} and total PUFA from 170 to 270 mg g^{-1} (Czesny et al., 2000).

The cultivated copepods in this study had low levels of essential fatty acids with high percentages of SFA and MUFA at the expense of the PUFA. The wild copepods had a DHA:EPA:ARA ratio of 18.5:10.4:1.0, probably due to a very diverse natural diet rich in DHA and EPA. The experimental larvae that had been fed with copepods had major percentages of DHA and EPA than the cultivated copepods themselves. One possible explanation is the manner of conducting Experiment 2, in which a daily supply of copepods nauplii, copepodits and adults and a mixture of 2 or 3 microalgae enabled copepods to feed and reproduce in the experimental tanks.

Larvae of Copepod and Mixed Treatments showed DHA:EPA:ARA ratios close to those of fat snook eggs (5.5:1.9:1.0 and 2.5:1.9:1.0, respectively), although the proportions of DHA were lower. Larvae fed exclusively with rotifers (Control Treatment) had low levels of DHA, and the DHA:EPA:ARA ratio (0.1:2.6:1.0) was therefore quite different from those of the other treatment groups. These data possibly explain why the Copepods and Mixed Treatments improved fat snook larval performance.

5. Conclusions

The use of the copepod *A. tonsa* as a first food for fat snook larvae improved larval development, even in the presence of small amounts of additives. These results show that the larvae fed with copepods had better essential fatty acid compositions than those fed with rotifers that are commonly used as the first food. However, more information on the optimum density of copepods for feeding *C. parallelus* larvae is still needed.

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