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**Research Article**

## **The copepod *Acartia tonsa* as live feed for fat snook (*Centropomus parallelus*) larvae from notochord flexion to advanced metamorphosis**

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**ABSTRACT.** From early development until the completion of metamorphosis, fat snook (*Centropomus parallelus*) larvae are commonly fed the rotifers *Brachionus* spp. and *Artemia* spp. nauplii. In this study, cultivated copepods *Acartia tonsa* were evaluated as feed for 15-to 45-day-old larvae. Two experiments were performed using twelve 30-L tanks stocked with 3.3 fat snook larvae L<sup>-1</sup>. Their initial mean weight and length were 1.35 ± 0.01 mg (mean ± standard deviation) and 3.83 ± 0.33 mm for 15-day-old larvae and 2.79 ± 1.2 mg and 6.99 ± 0.88 mm for 31-day-old larvae. Three dietary treatments were carried out in four replicates, including Rotifer (R), *Artemia* (A) and/or Copepod (C). Experiment 1 included Diet RA (control), Diet RC and Diet RCA; while experiment 2 included Diet A (control), Diet C and Diet AC. The survival and growth of larvae fed the Diet RCA in experiment 1 were significantly higher than the others. In experiment 2, the inclusion of copepods in the diet did not improve survival and growth, however, larvae fed Diet C had the highest DHA/EPA ratio. We conclude that the copepod *Acartia tonsa* provides an important nutritional benefit to fat snook larvae undergoing metamorphosis.

**Keywords:** *Centropomus parallelus*, snook, copepod, hatchery, fatty acids, lipids, marine fish.

### **INTRODUCTION**

Snooks, *Centropomus* spp., are euryhaline marine fishes that inhabit coastal waters in tropical and subtropical regions of the Americas (Rivas, 1986). Because snooks have high commercial value (Cerqueira, 2005) they are caught in large quantities by coastal fishermen, often before sexual maturation (Chaves, 1963; Rojas, 1975). Overfishing has contributed to the decline of their natural stocks (Muller & Taylor, 2012; Ley & Allen, 2013). For this reason, aquaculture can help relieve fishing pressure.

In marine fish hatcheries, larvae are commonly fed with live rotifers *Brachionus* spp. and *Artemia* spp. nauplii. However, both are deficient in highly unsaturated fatty acids (HUFA), which are essential to the healthy development of the larvae, and require enrichment with lipid emulsions prior to use (Lavens &

Sorgeloos, 1996; Stottrup *et al.*, 1999; Knuckey *et al.*, 2005).

*Artemia* is widely used during the notochord post-flexion stage in marine fish hatcheries due to their ease of storage in the form of cysts, ease of handling, and suitability for bioencapsulation, but its costs are high (Sorgeloos *et al.*, 1986; García *et al.*, 2008a). Additionally, when enriched with HUFA, *Artemia* mainly accumulates triglycerides, whose fatty acids are less bioavailable for use by fish larvae (Sargent *et al.*, 1999b; Sorgeloos *et al.*, 2001; Schipp, 2006). Therefore, co-feeding with copepods may be an interesting nutritional alternative at this stage of larviculture.

In the wild, copepods are the main food source of marine fish larvae, and are nutritionally superior than rotifers and *Artemia* (Stottrup & Norsker, 1997; Stottrup *et al.*, 1999; El-Sabaawi *et al.*, 2009). Calanoid

copepods have been used experimentally as live feed in marine fish hatcheries and are found to improve survival and growth (Schipp *et al.*, 1999; Schipp, 2006; Stottrup, 2006; Olivotto *et al.*, 2008; Russo *et al.*, 2009) and even stress resistance (Costa *et al.*, 2015). Copepods are a rich source of natural antioxidants and phospholipids, such as HUFA, and according to Stottrup (2003) provide a good ratio of docohexaenoic and eicosapentaenoic acids (DHA/EPA = 2).

In the first months of life, fat snook (*Centropomus parallelus*) larvae undergo drastic morpho-physiological changes in the process of metamorphosis until they become juveniles (Alvarez-Lajonchère *et al.*, 2002), and during laboratory rearing important losses occur, probably due to inadequate diets and environmental conditions (Cerqueira & Tsuzuki, 2009).

In a previous study with fat snook *C. parallelus* (Barroso *et al.*, 2013), the use of the copepod *Acartia tonsa* as first feed for yolk-sac larvae, even in low densities, increased survival and growth, until 14 days after hatching (notochord flexion). Therefore, this study tested if the same approach could be applied for older fat snook larvae, from the notochord post-flexion (15 days-old larvae) to an advanced stage close to metamorphosis (45 days-old larvae). In addition, the nutritional quality of the copepods, including the fatty acids content, was compared with rotifers and *Artemia*.

## MATERIALS AND METHODS

### Fish and holding facilities

This study was conducted at the Laboratório de Piscicultura Marinha of the Universidade Federal de Santa Catarina (UFSC). The experiments followed the procedures of UFSC's Ethics Committee on the Use of Animals (Protocol PP00861).

Snook larvae, obtained by induced spawning of cultivated fat snook (*Centropomus parallelus*) adults (Ferraz *et al.*, 2002), were reared in a cylindrical 5 m<sup>3</sup> tank (Alvarez-Lajonchère *et al.*, 2002) at an initial stocking density of 50 hatched larvae L<sup>-1</sup>. They were fed rotifers (*Brachionus rotundiformis*) from day 3 to day 25 after hatching and brine shrimp nauplii (*Artemia franciscana*) from day 20 to day 30 after hatching.

### Plankton culture

Microalgae were produced according to the method described by Lourenço (2006) and were used at a density of 70×10<sup>4</sup> cells mL<sup>-1</sup> for *Chaetoceros muelleri* and *Isocrysis galbana*, and 100×10<sup>4</sup> cells mL<sup>-1</sup> for *Nannochloropsis oculata*.

Copepods were collected from the wild and isolated as described by Barroso *et al.* (2013). Cultivation

methods were adapted from Bersano (2003). The copepods were raised in 500 L cylindrical tanks at a salinity of 35 g L<sup>-1</sup> and temperature of 26°C. They were fed *C. muelleri* and *I. galbana* at 30×10<sup>4</sup> cells mL<sup>-1</sup> and 60×10<sup>4</sup> cells mL<sup>-1</sup>, respectively and cultured in a 7 day batch system. The nauplii were reared under the same feeding conditions, requiring eight days to become adults. These cultures were always begun in small containers (5 L), and the quantity of their rations and the volume of their tanks gradually increased up to 40 L, based on population growth. The same process was used in the production tanks (250 L). Samples representing the whole population (nauplii, copepodites and adults) in the production tanks were used to feed fat snook larvae.

Rotifer *B. rotundiformis* were maintained at a controlled water temperature of 28°C, with salinity of 25 g L<sup>-1</sup>, in small culture containers (2 L) and fed *Nannochloropsis oculata* at a density of 100×10<sup>4</sup> cells mL<sup>-1</sup> for seven days. As their density increased, the rotifers were shifted to a 40 L and then to a 500 L cylinder-conical tank with constant aeration, where they were fed with dry yeast (*Saccharomyces cerevisiae*; 1 g 10<sup>-6</sup> rotifers), microalgae *N. oculata* (100×10<sup>4</sup> cells rotifer<sup>-1</sup>), and Culture Selco (INVE®, Belgium; 0.5 g 10<sup>-6</sup> rotifers, day 3 only). Once the rotifers reached a density of 500 ind mL<sup>-1</sup>, they were used in the larval feeding experiments.

Cysts of *A. franciscana* (*Artemia* High 5, INVE®, Belgium) were incubated for around 18 h in 500 L cylinder-conical tanks according to established protocols (Lavens & Sorgeloos, 1996). Hatched nauplii were reared with a lipid emulsion (DC-DHA Selco, INVE®, Belgium) at a water temperature of 28°C, and a stocking density of 500,000 nauplii L<sup>-1</sup> for 24 h, to obtain metanauplii enriched with highly unsaturated fatty acids.

### Larviculture trials

Two experiments were conducted with snook larvae of different ages in 12 rectangular 30 L tanks to test different feeding regimes. The larvae were transferred from the 5 m<sup>3</sup> larviculture tank with a 2 L beaker to the 30 L tanks. The larvae were reared in a clear water system, with gentle aeration, water temperature around 26°C and daily water exchange of 10 to 25% of the tank volume. The stocking density was 3.3 larvae L<sup>-1</sup>.

At the end of both experiments, all fish were previously anaesthetized with benzocaine (40 mg L<sup>-1</sup>), measured to the nearest mm in total length, and weighed to the nearest 1 mg. Survival, growth, condition factor (K) and specific growth rate (SGR) by weight (% day<sup>-1</sup>) were evaluated. K and SGR was calculated by:

$$K = (W/L^3) \times 100$$

SGR =  $100 \times [\ln(\text{final } W - \text{initial } W) / \text{time (days)}]$ ,  
where  $W$  = weight (mg) and  $L$  = length (mm).

### Experiment 1

Larvae in the nothocord flexion stage, 15 days after hatching, with initial wet weight and total length of  $1.35 \pm 0.01$  mg (mean  $\pm$  SD) and  $3.83 \pm 0.33$  mm, respectively, were reared for 12 days (until 27 days after hatching). Three treatments or feeding regimes were tested with four replicates, including Rotifer (R), *Artemia* (A) and/or Copepod (C). Diet RA, the control treatment, included: rotifers ( $5 \text{ mL}^{-1}$ ) and *Artemia* ( $0.25 \text{ mL}^{-1}$ ); Diet RC: rotifers ( $5 \text{ mL}^{-1}$ ) and copepods ( $0.25 \text{ mL}^{-1}$ ); and Diet RCA: rotifers ( $5 \text{ mL}^{-1}$ ), copepods ( $0.12 \text{ mL}^{-1}$ ) and *Artemia* ( $0.12 \text{ mL}^{-1}$ ). All feed items were given simultaneously and their concentration in the tanks was evaluated each morning, and new preys were added, when needed, according to the established treatments. Temperature ( $27.3 \pm 1.0^\circ\text{C}$ ), salinity ( $35.0 \pm 1.0 \text{ g L}^{-1}$ ) and dissolved oxygen ( $5.6 \pm 0.5 \text{ mg L}^{-1}$ ) were also monitored daily.

### Experiment 2

Larvae, 31 days after hatching, with initial wet weight and total length of  $2.79 \pm 1.2$  mg and  $6.99 \pm 0.88$  mm, respectively, were reared for 14 days (until 45 days after hatching). Three treatments with four replicates were tested. Diet A (the control treatment): *Artemia* ( $1.0 \text{ mL}^{-1}$ ); Diet C: copepods ( $0.2 \text{ mL}^{-1}$ ); Diet AC: *Artemia* ( $0.5 \text{ mL}^{-1}$ ) and copepods ( $0.1 \text{ mL}^{-1}$ ). The amount of copepods was based on previous studies (Russo *et al.*, 2009; Barroso *et al.*, 2013), to provide the minimum nutrient requirement for larva of this age, mainly of essential fatty acids. The concentration of live feed remaining in the tanks was monitored each morning. Since the values found were always very close to zero, new preys were added according to the established treatments. Temperature ( $27.0 \pm 2.1^\circ\text{C}$ ), salinity ( $35.0 \pm 1.0 \text{ g L}^{-1}$ ), and dissolved oxygen ( $5.8 \pm 0.9 \text{ mg L}^{-1}$ ) were monitored daily.

### Biochemical analysis

Samples of eggs, zooplankton and 15, 27, 30 and 45 days-old fat snook larvae were collected from the different treatment tanks and stored in sealed glass tubes at  $-80^\circ\text{C}$  for 24 h after the last feeding. Analyses were performed at the Laboratory of Fats and Oils at Embrapa Food Technology, RJ. Lipids were extracted according to the method of Bligh & Dyer (1959), using 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. Fatty acid methyl esters (FAME) were prepared according to the method of

Hartman & Lago (1973). Gas chromatography was performed using an Agilent 6890 chromatograph fitted with a cyanopropylsiloxane capillary column ( $60 \text{ m} \times 0.32 \text{ mm} \times 0.25 \text{ mm}$ ).

The results of fatty acid methyl esters were expressed as weight percentages (area of normalization) of the identified FAME. The components were identified by comparing the retention times with reference standards (Nu-Chek Prep® Inc., Elysian, USA), and the standard marine-source of polyunsaturated fatty acids (PUFA) PUFA 3 Menhaden oil (Supelco®, Bellefonte, USA). The standard deviation could not be calculated due to the reduced amount of material samples, although they matched the fatty acids of larvae and juvenile fat snook analyzed in earlier studies with this species (Barroso, 2010) and in experiments with 14 days-old fat snook larvae (Barroso *et al.*, 2013).

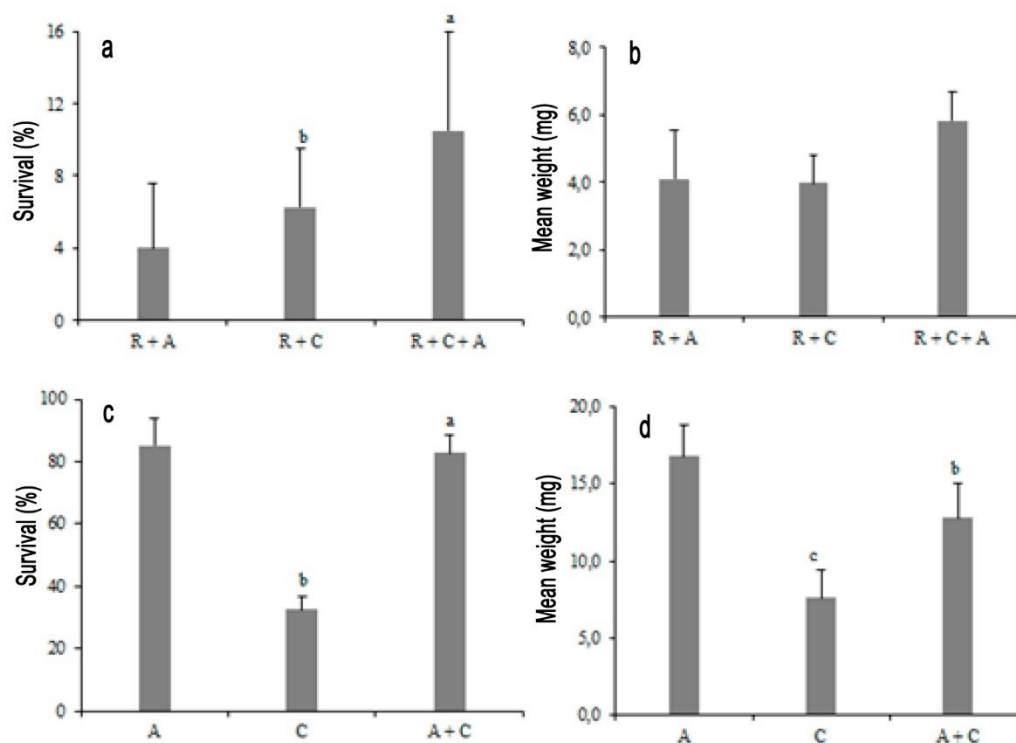
### Statistical analysis

Statistical analysis of variance was performed on all of the evaluated larval parameters (*i.e.*, survival, weight, total length, condition factor and specific growth rate) after confirmation of the homoscedasticity of variances and normality of data distribution. Mean and standard deviation were determined for each variable. Where significant differences between treatments ( $\alpha = 0.05$ ) were detected, the Tukey HSD test for comparing means was applied. All tests were performed using GENES software (Cruz, 2013).

## RESULTS

In Experiment 1, larvae fed the Diet RCA (Figs. 1a-1b) had a significantly higher ( $P < 0.05$ ) survival ( $10.5 \pm 5.5\%$ ) and mean weight ( $5.83 \pm 0.87$  mg) than those fed the Diet RA ( $4.0 \pm 3.6\%$  and  $4.10 \pm 1.42$  mg) or the diet RC ( $6.2 \pm 3.3\%$  and  $3.96 \pm 0.82$  mg). The RCA treatment as well as the control (RA) also had higher values for the condition factor and specific growth rate, both respectively (Table 1), than the RC treatment. There was no difference in total length among the treatments. Additionally, only larvae that ingested Diet RCA were strongly pigmented, which is a good biological indicator for marine fish larvae. Moreover, these larvae had completed the notochord flexion phase and their pectoral and caudal fins were in advanced development. The first and second dorsal fins were not differentiated and the pelvic and anal fins were in early development.

In the fatty acid profile of the zooplankton (Table 2), the copepods and enriched *Artemia* had higher amounts of palmitic acid (C16:0) and, therefore, higher levels of saturated fatty acids than the rotifers. The palmitoleic



**Figure 1.** Survival and weight (mean  $\pm$  SD) of 27 days-old (Experiment 1: 1a, 1b) and 45 days-old (Experiment 2: 1c, 1d) *Centropomus parallelus* larvae fed different diets. Rotifer (R), *Artemia* (A), Copepod (C).

**Table 1.** Total length (TL) (mean  $\pm$  SD), condition factor (K) and specific growth rate (SGR) of 27 days-old (Experiment 1) and 45 days-old (Experiment 2) *Centropomus parallelus* larvae fed different diets. Rotifer (R), *Artemia* (A), Copepod (C). Significant differences are denoted by different superscript letters ( $P < 0.05$ ).

|                            | Experiment 1 diets            |                              |                               | Experiment 2 diets            |                               |                               |
|----------------------------|-------------------------------|------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
|                            | RA                            | RC                           | RCA                           | A                             | C                             | AC                            |
| TL (mm)                    | 7.79 $\pm$ 1.50 <sup>a</sup>  | 8.70 $\pm$ 1.43 <sup>a</sup> | 8.80 $\pm$ 1.60 <sup>a</sup>  | 12.98 $\pm$ 1.77 <sup>a</sup> | 10.14 $\pm$ 1.41 <sup>b</sup> | 9.62 $\pm$ 2.09 <sup>b</sup>  |
| K (%)                      | 0.95 $\pm$ 0.40 <sup>a</sup>  | 0.52 $\pm$ 0.23 <sup>b</sup> | 0.81 $\pm$ 0.37 <sup>a</sup>  | 1.25 $\pm$ 0.44 <sup>a</sup>  | 0.71 $\pm$ 0.30 <sup>b</sup>  | 1.23 $\pm$ 0.54 <sup>a</sup>  |
| SGR (% day <sup>-1</sup> ) | 9.98 $\pm$ 4.35 <sup>ab</sup> | 8.27 $\pm$ 3.99 <sup>b</sup> | 11.52 $\pm$ 4.65 <sup>a</sup> | 13.14 $\pm$ 3.38 <sup>a</sup> | 6.97 $\pm$ 3.98 <sup>c</sup>  | 10.47 $\pm$ 4.27 <sup>b</sup> |

and oleic acids (C16:1 and C18:1) in the rotifers totaled 455 mg g<sup>-1</sup> of monounsaturated fatty acid (MUFA). Copepods and *Artemia* were abundant in DHA and EPA, but the copepods offer higher availability (60% and 43%, respectively). Arachidonic acid (ARA) values were similar for copepods and *Artemia*, and higher for rotifers. The DHA/EPA ratio was 1.8 for copepods, 2.5 for *Artemia* and 0.05 for rotifers, whereas EPA/ARA was 10.0, 3.4 and 2.6, respectively. Rotifers had a very low DHA value, resulting in disproportionate fatty acid content. *Artemia* had the highest amount of linolenic acid (18:3n-3).

The DHA/EPA ratio of fat snook eggs is around 5.0, while the EPA/ARA ratio is approximately 2.5. All of the larvae 27 days after hatching exhibited similar SFA composition, which predominantly comprised palmitic

and stearic acids (C16:0 and C18:0). Regarding MUFA, the larvae fed Diet RCA had a higher amount of palmitoleic acid (C16:1). The amount of linolenic acid (C18:3n-3) was higher in the larvae fed Diet RA compared to the other treatments.

In Experiment 2, survival was significantly higher ( $P < 0.05$ ) for 45 days-old larvae fed diets A (85.3  $\pm$  8.8%) and AC (82.8  $\pm$  5.6%), compared to those in which just copepods were used (32.8  $\pm$  4.3%, Fig. 1c). In terms of growth, the highest mean weight (Fig. 1d) was found in larvae fed Diet A. Moreover, the condition factor was significantly higher ( $P < 0.05$ ) in larvae fed diets AC and A than those fed Diet C, while the highest specific growth rate and total length were in larvae fed Diet A (Table 1).

**Table 2.** Fatty acid composition (mg g<sup>-1</sup> dry weight) of zooplankton, eggs and initial and final *Centropomus parallelus* larvae, in two experiments, fed different diets: Rotifer (R); *Artemia* (A); Copepod (C). 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. Values in each column represent two or three replicates/analysis. The unidentified peaks were not considered.

| Fatty acids          | Copepods | Rotifers | Artemia | Fat           | Experiment 1        |                |       |       | Experiment 2        |                |       |       |
|----------------------|----------|----------|---------|---------------|---------------------|----------------|-------|-------|---------------------|----------------|-------|-------|
|                      |          |          |         | snook<br>eggs | Larvae<br>(initial) | Larvae (final) |       |       | Larvae<br>(initial) | Larvae (final) |       |       |
|                      |          |          |         |               |                     | RA             | RC    | RCA   |                     | A              | C     | AC    |
| C14:0                | 57.3     | 20.5     | 21.2    | 26.7          | 19.3                | 14.9           | 19.9  | 16.4  | 19.3                | 8.6            | 19.4  | 8.5   |
| C16:0                | 207.2    | 125.2    | 221.6   | 190.0         | 260.8               | 226.5          | 234.2 | 214.0 | 245.2               | 187.4          | 235.0 | 181.2 |
| C18:0                | 62.2     | 56.5     | 87.7    | 41.2          | 113.9               | 117.4          | 100.1 | 87.0  | 119.3               | 91.6           | 108.1 | 101.7 |
| ∑ SFA <sup>a</sup>   | 351.2    | 234.1    | 367.2   | 267.1         | 431.0               | 395.1          | 368.1 | 322.4 | 406.7               | 308.6          | 376.9 | 311.3 |
| C16:1(n-7)           | 47.5     | 162.6    | 29.3    | 52.8          | 69.5                | 4.4            | 24.8  | 64.1  | 5.8                 | 5.1            | 26.0  | 4.0   |
| C18:1(n-9)           | 101.0    | 247.3    | 179.3   | 168.6         | 135.2               | 214.2          | 181.2 | 164.7 | 177.7               | 214.3          | 128.0 | 185.0 |
| ∑ MUFA <sup>b</sup>  | 161.0    | 454.5    | 274.6   | 239.2         | 216.6               | 283.7          | 242.0 | 286.5 | 256.8               | 228.1          | 162.8 | 197.5 |
| C18:2(n-6)           | 43.6     | 30.8     | 55.2    | 92.4          | 43.6                | 64.4           | 76.0  | 84.1  | 61.6                | 69.8           | 31.1  | 59.8  |
| C20:4(n-6) ARA       | 9.2      | 24.0     | 12.3    | 19.9          | 47.4                | 22.0           | 22.1  | 37.3  | 35.9                | 26.4           | 34.1  | 31.5  |
| C18:3(n-3)           | 33.9     | 8.2      | 143.8   | 21.2          | 4.5                 | 105.0          | 10.8  | 32.8  | 40.4                | 134.6          | 30.4  | 116.9 |
| C20:5(n-3) EPA       | 95.8     | 62.5     | 41.6    | 46.8          | 72.8                | 45.6           | 52.4  | 78.3  | 58.6                | 43.4           | 61.8  | 47.7  |
| C22:5(n-3)           | 6.9      | 31.8     | 5.4     | 29.3          | 64.5                | 20.0           | 24.3  | 38.2  | 43.5                | 9.6            | 20.1  | 13.1  |
| C22:6(n-3) DHA       | 170.3    | 3.4      | 102.0   | 226.6         | 61.6                | 44.7           | 169.7 | 94.6  | 51.1                | 111.2          | 247.8 | 164.3 |
| ∑ PUFA <sup>c</sup>  | 359.7    | 160.7    | 363.0   | 436.2         | 294.4               | 301.7          | 355.3 | 365.3 | 294.1               | 395.1          | 425.3 | 433.3 |
| ∑ Trans <sup>d</sup> | 65.9     | 95.7     | 13.9    | 20.3          | 19.3                | 18.1           | 22.9  | 24.4  | 19.9                | 67.3           | 35.1  | 58.0  |
| DHA/EPA              | 1.8      | 0.05     | 2.5     | 4.8           | 0.9                 | 1.0            | 3.2   | 1.2   | 0.9                 | 2.6            | 4.0   | 3.4   |
| EPA/ARA              | 10.4     | 2.6      | 3.4     | 2.4           | 1.6                 | 2.1            | 2.4   | 2.1   | 1.6                 | 1.6            | 1.8   | 1.5   |

In relation to fatty acids (Table 2), larvae fed Diet A in Experiment 2 had a similar profile to that obtained with Diet AC. The differences in the Diet A treatment were the amount of linolenic acid (C18:3n-3), which was 15% higher, and DHA, which was 32% lower. The fat snook larvae at the beginning of Experiment 2 had greater SFA availability regarding PUFA (30% less), due to the lower amounts of DHA. The MUFA content was similar for both eggs and initial larvae. In comparison, the eggs and final larvae of all treatments had high PUFA levels (~400 mg g<sup>-1</sup>). The larvae given Diet C had a higher amount of DHA (247.8 mg g<sup>-1</sup>) with a DHA/EPA ratio of 4.0. In those fed Diet AC, good amounts of palmitic, stearic, oleic acids, DHA, EPA, and ARA were found in the larvae, with a good DHA/EPA ratio of 3.4.

## DISCUSSION

At the end of Experiment 1, growth and survival obtained with Diet RCA were significantly higher, indicating that the addition of copepods increased larval development, as corroborated by advanced morphological changes and strong pigmentation. In a study of dusky grouper (*Epinephelus marginatus*), larviculture in 60 m<sup>3</sup> tanks fed wild zooplankton, rotifers and *Artemia* (Russo *et al.*, 2009), even at low densities

(<0.4 mL<sup>-1</sup>), copepods were actively selected, enhancing the quality of diet and then determining satisfactory survival rates. Pigmentation is also an important parameter for marine fish larvae, indicating good health and nutrition, since copepods are also a source of vitamins C and E, antioxidants, astaxanthin and polar lipids, which are more available to fish larvae than triglycerides (Sargent *et al.*, 1999a; Stottrup, 2003).

One of the reasons for the better performance with Diet RCA may have been the combination of the nutritional content of the different live feed offered, providing a good relationship between the essential fatty acids. Regarding PUFA, the larvae in the Diet RA contained 105 mg g<sup>-1</sup> of linolenic acid, which probably originated from the ingestion of *Artemia*. The larvae in the Diet RC had higher amounts of DHA, with the highest DHA/EPA ratio (3.2), similar to values found in other marine fish larvae (Izquierdo *et al.*, 2000). The amount of ARA obtained was also within the standards described in the literature (Garcia *et al.*, 2008b), indicating that Diet RC had a good balance between the essential fatty acids. When comparing these final contents with the initial larvae (15 days-old), it was found that in this treatment larvae assimilated dietary DHA, probably from the copepods, which have high DHA content. Linolenic acid (C18:3n3) content found

in larvae fed Diet RA was also very high. It comes probably from the lipid emulsion used to enrich the *Artemia* nauplii, which naturally bioencapsulate substances and also contain large amounts of this fatty acid (Ladhar *et al.*, 2014). This relative abundance of linolenic acid could be a problem, since marine fish larvae preferentially hydrolyze other fatty acids (Plante *et al.*, 2007), with a limited ability to biosynthesize phospholipids (Sargent *et al.*, 1999b). The control group also displayed an unbalanced ratio between PUFA's, with limited availability of DHA.

The proportion of essential fatty acids found in larval tissues is directly related to growth (Izquierdo *et al.*, 2000) and the DHA levels should be at least twice as high as EPA levels (Sargent *et al.*, 1999a; Stottrup, 2003). Experiment 1 showed that the addition of copepods in co-feeding with rotifers and *Artemia* improved development of young larvae (<30 days-old). Despite the fact that copepods were offered at low densities, they were an excellent source of DHA, an extremely important essential fatty acid for marine fish larvae. The inclusion of the copepod *A. tonsa* in the diet of *Centropomus undecimalis* larvae, co-feeding with rotifer, not only increased growth but also resistance to heat stress (Costa *et al.*, 2015), which is a very interesting nutritional effect.

In experiment 2, older larvae (31 to 45 days after hatching) fed the diets A and AC had significantly higher survival and condition factors than larvae fed Diet C, but weight, total length and the specific growth rate was higher only in the control treatment (*Artemia*). An explanation for this is that the *Artemia* treatment provided a higher feed density (100%) than Diet C (20%) and Diet AC (10% copepod plus 50% *Artemia*).

Comparing the fatty acid content of initial and final larvae in experiment 2, all of the treatments provided high levels of PUFA. Therefore, at the end of the experiment, larvae had a better nutritional condition in relation to essential fatty acids than at the beginning.

The larvae fed Diet C had a good fatty acids profile, a DHA/EPA ratio (4.0) considered excellent for marine fish (Sargent *et al.*, 1999a), and good survival, but their development was significantly lower than in the *Artemia* treatment, as a consequence of less feed available. The fatty acids profile of larvae fed Diet A had a good PUFA balance compared to larvae at the beginning. However, the larvae fed copepods (Diet C and Diet AC) had the highest DHA contents.

The copepods were nutritionally beneficial to the development of the 31 to 45 days-old larvae, but due to the high quantity of live feed required in this stage, it is understood that the enriched *Artemia* nauplii provided better results in terms of survival and growth. Never-

theless, further studies are necessary to determine the optimal increase in the percentage of copepods in co-feeding with *Artemia*.

## CONCLUSIONS

The use of copepod *Acartia tonsa* as a complementary dietary source enhanced growth and survival of 15 to 27 days-old larvae. For 30 to 45 days-old larvae, the provision of copepods did not improve growth and survival, but benefitted the larvae's fatty acids profile by increasing DHA content, despite the small amount added. The inclusion of this live feed is recommended as a complementary diet during fat snook larviculture, as methods for mass cultivation of copepods are still in development.

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