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ARTICLE

Use of Copepods during Early Feeding Stages of Spotted Rose Snapper Larvae in a Scale-Up System

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Abstract
Growth but not survival of Spotted Rose Snapper Lutjanus guttatus larvae was improved when calanoid copepods Pseudodiaptomus euryhalinus were included as early feed in a pilot-scale production system. Production of P. euryhalinus that were fed microalgae Nannochloropsis oculata was stable through the snapper spawning season (May–September; 21.4–29.5°C), providing 6,200–9,400 copepods/L with higher nutritional quality than rotifers Brachionus rotundiformis. To evaluate the influence of including P. euryhalinus as a first feed, three dietary treatments were administered to larvae from 3 to 15 d posthatch: (1) P. euryhalinus only, (2) a mix of both P. euryhalinus and B. rotundiformis, and (3) B. rotundiformis only. Use of P. euryhalinus as live feed produced significant differences in larval specific growth rate, which was 48.4% of body weight (BW)/d for treatment 1; 17.5% BW/d for treatment 2; and 7.3% BW/d for treatment 3. However, survival was significantly higher for Spotted Rose Snapper larvae that received B. rotundiformis as feed; survival was 5.2% for treatment 3, whereas it was 2.2% for treatment 2 and 1.4% for treatment 1. Larvae that were given P. euryhalinus as feed also had better biochemical content than larvae that were fed only B. rotundiformis.

Rotifers and brine shrimp Artemia spp. have been the main live-feed types used in fish larviculture because they are readily available. In the wild, copepods are an abundant food source for marine fish and are highly nutritional and easily digestible prey (Ajiboye et al. 2011; Drillet et al. 2011). Incorporation of copepods as a first feed for fish larvae has been shown to enhance growth and survival and to reduce the incidence of disease and deformities (Støttrup 2000). Species belonging to the Lutjanidae family (snappers) have high commercial value and good aquaculture potential (Leu et al. 2003; Ogle and Lotz 2006), but the survival of lutjanids during captive production has been low, mainly because the small gape size of the larvae restricts their prey size to 50–100 μm (Yúfera and Pascual 1984). A diet of rotifers resulted in 0% survival of Mangrove Red Snapper Lutjanus argentimaculatus (Emata et al. 1999), but the inclusion of copepod nauplii increased the survival and growth rate of Red Snapper L. campechanus (Ogle and Lotz 2000) and Spotted Rose Snapper L. guttatus (Boza-Abarca et al. 2008). There is still room for improvements in current methods of copepod culture, as high production requires large volumes. In 2000, the Centro de Investigación en Alimentación y Desarrollo–Mazatlán (CIAD–Mazatlán) began developing culture technology for the Spotted Rose Snapper, and improvements in survival and growth have since been achieved. The copepods Pseudodiaptomus euryhalinus and Tisbe monozota were successfully included in first-feeding diets for Spotted Rose Snapper (García-Ortega et al. 2005; Abdo-de la Parra et al. 2010). Stomach contents of the larvae included P. euryhalinus adults and nauplii, indicating that

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the larvae were able to ingest all life stages of the copepod. To fulfill larval requirements, the volume of tanks used for copepod production was increased from 300 to 1,000 L (Puello-Cruz et al. 2006) and ultimately to 7,000 L. Our objective in the present study was to evaluate this easy scale-up as a practical method for increasing copepod production and to assess the growth and survival of Spotted Rose Snapper larvae when *P. euryhalinus* were included in the diet.

**METHODS**

**Culture of Microalgae**

Strains of the microalgae *Nannochloropsis oculata* and *Isochrysis* sp. were obtained from the facilities of CIAD–Mazatlán. Microalgae were cultivated in F/2 medium (Guillard 1975) prepared with seawater (salinity = 34 psu) that had been filtered through 10- and 2-µm cartridges, ultraviolet (UV) sterilized, placed in 2-L flasks, and kept at 20°C. Culture volumes were increased to 20-L, 80-L, and 700-L indoor tanks maintained at 23°C, with a salinity of 34 psu, a 24-h light regime (Philips fluorescent bulbs, ballast type, 40 W), and strong aeration. After 7 d of culture, the microalgae were pumped (Evans AQUA45W waterproof pump) into a 7,000-L, outdoor, conical white fiberglass tank (3-m diameter × 1.5-m deep) that was covered with black mesh to achieve 30% light reduction; an air stone was placed at the bottom of the tank for aeration.

**Copepod Culture**

*Pseudodiaptomus euryhalinus* were obtained from the continuous pure cultures maintained at the Nutrition Laboratory of CIAD–Mazatlán. The outdoor copepod culture had an initial volume of 2,000 L in a 7,000-L, round white fiberglass tank; over a period of approximately 2 months, seawater and microalgae were added (according to cell count, to maintain >320 cells/µL; Velasco-Blanco et al. 2011) until a culture volume of 6,000 L was reached. A new and separate copepod culture was started every fifth day in a 1,000-L, conical white fiberglass tank by stocking with 1,000 copepods/L. After 15 d, the culture was then added to the 7,000-L tank. The tank water was fertilized with Bayfolan (3.75 g/100 L of seawater). *Nannochloropsis oculata* were added at an initial concentration of 4 × 10¹¹ cells/mL during the exponential phase. During the experiment, water characteristics were maintained at the following levels: salinity at 30.9 psu (SD = 1.3), temperature at 21.4–29.5°C, dissolved oxygen at 8.6–6.9 mg/L (saturation = 80.0%, SD = 7; measured daily with a YSI 85 multimeter; YSI, Yellow Springs, Ohio), and NH₃ below 0.05 mg/L (Nutrafin test; Rolf C. Hagen, Canada). Every third day, approximately 2 L of water were drained from the bottom of each 1,000-L tank to eliminate any accumulation of organic wastes. The copepod production procedure was carried out continuously for 5 months during the spawning period of Spotted Rose Snapper (May–September). Each partial harvest of copepods (500 L/d) was accompanied by depuration, a process by which the copepods were held in tanks of clean seawater, filtered through a 5-µm cartridge (Hytrex ii), and UV treated with gentle aeration, renewing the water at least three times, to reduce microbial contaminants as described by Puello-Cruz et al. (2006). All sizes and stages of copepods (Puello-Cruz et al. 2008) were concentrated and stocked into a 300-L tank with filtered, sterilized seawater. Microalgae from indoor cultures were added to the 300-L tanks 2 h before the copepods were given to first-feeding Spotted Rose Snapper larvae in the pilot-system tanks. Three aliquots of 100 mL each were examined under a Leica MZ2 dissecting microscope in order to count and differentiate the copepod stages.

**Culture of Rotifers**

Continuous mass production of *Brachionus rotundiformis* occurred in 7,000-L culture tanks with intense aeration; the rotifers were fed *N. oculata* (≥20 × 10⁶ cells/mL). Rotifers were cleaned and washed before being fed to the Spotted Rose Snapper larvae (Alvarez-Lajonchère et al. 2012).

**Larval Experiment**

Spotted Rose Snapper were obtained from naturally spawning broodstock (F₁) reared at CIAD–Mazatlán. Twelve 3,000-L, round tanks (2 m diameter × 1 m deep) with white bottoms and black sides were placed outdoors in an area covered with mesh to obtain 30% light reduction. Each tank initially contained a 1,000-L volume of filtered, sterilized seawater, into which 21.5 mL of fertilized eggs (~65.9 eggs/L) were stocked. The water volume in the tank increased when microalgae were added. To evaluate the larval hatch percentage at 24 h and larval survival at 48 h (1 d posthatch [dph]), four 1-L samples of water were taken from different parts of the tank, and the numbers of live and dead larvae and unhatched eggs in each sample were counted. Percent hatch was determined as

\[
\% \text{ hatch} = \frac{(\text{live larvae} + \text{dead larvae})}{(\text{live larvae} + \text{dead larvae} + \text{unhatched eggs})} \times 100.
\]

Throughout the experimental period, temperature, salinity, and dissolved oxygen were measured daily in all 12 tanks by using a YSI 85 multimeter; ammonia and nitrite in each tank were also measured daily by use of test kits (Instant Ocean, Aquarium Systems, Mentor, Ohio). Two air stones were placed at the bottom of each tank for aeration. Every evening, tanks were individually covered with black mesh (30% light reduction) from 1700 to 0800 hours to prevent bird predation from occurring overnight. The outlet in the center of the tank was covered with nylon mesh (150 µm), and the aeration hosepipes were cleaned every morning before live feed was added. The green water technique was applied by adding the required volume of microalgae (*N. oculata* at 5 × 10⁵ cells/mL; *Isochrysis* sp. at 5 × 10⁵ cells/mL) from the microalgae culture tanks to the larval tanks via a plastic pipe connected to a waterproof pump (Evans AQUA45W pump, 60 Hz, 18.0 L/min). Three live-feed treatments at three stocking densities were applied from 3 to 15 dph: (1) *P. euryhalinus* at
1,000 copepods/L; (2) a mix of P. euryhalinus at 500 copepods/L and B. rotundiformis at 5,000 rotifers/L; and (3) B. rotundiformis at 10,000 rotifers/L. Differences in prey density were based on the dry weight of the organisms, with copepod nauplii being approximately five times heavier (1.67 μg; Mones-Saucedo 2011) than rotifers (0.29 μg; Piña-Valdez 2004). Four replicates of each live-feed treatment were carried out through 15 dph. Water in larval tanks was not renewed during the experiment. Daily live feed was counted for each tank (three 500-mL samples taken from different parts of the tank); microalgae were counted with a hemocytometer, and rotifers and copepods were counted with a dissecting microscope (Leica MZ6). Adequate daily rations of food in accordance with the different treatments were maintained. Ten larvae were removed from each tank every third day through 15 dph and were anesthetized with 2-phenoxethanol at 200 μL/L (Sigma Aldrich, Toluca, Mexico) for measurements using either a dissecting microscope equipped with an ocular micrometer or an electronic vernier caliper (precision = 0.001 mm) depending on larval size. The larvae were rinsed in distilled water and dried with a cotton cloth to eliminate water and salt, placed on pre-weighed foil trays, and then weighed on a microbalance (Mettler MT5, Mettler Instruments, Hightstown, New Jersey). At the end of the experiment, the samples were rinsed with distilled water before being placed in a freeze dryer and finally stored in a freezer at −80°C.

**Biochemical Analyses**

Protein was measured with a nitrogen analyzer (Flash 2000 organic elemental analyzer; Thermo Scientific; Method 990.03, AOAC 1984). The total fatty acid (FA) composition was determined by using a Foss Tecator Soxtec 2050 (Method 920.39, AOAC 1984).

**Analysis of fatty acids.**—Fatty acid composition was determined by gas chromatography (Varian gas chromatograph; Varian, Palo Alto, California). For lipid extraction (Folch et al. 1957), 2 g of the sample were mixed in a homogenizer with 10 mL of methanol and 20 mL of chloroform. The mixture was filtered and placed in a 500-mL separation funnel. The extraction process was repeated three times. The filtered solution was mixed with 0.88% KCl to form two phases, with the lower layer corresponding to lipids, and it was separated and purified with a 1:1 mix of methanol: distilled water. The remaining chloroform was evaporated in a water bath with nitrogen flow to extract the lipids.

Fatty acids were methylated (Method 969.33, AOAC 1988). Each lipid sample, along with 10 mL of NaOH at 0.5 N, 12 mL of BF₃ at 14%, and 4 mL of n-heptane, was placed in a 100-mL, round-bottom flask connected to a reflux column for 5–10 min. The solution was then mixed with a saturated solution of NaCl to produce two phases. The superior layer of n-heptane was transferred to a test tube with Na₂SO₄ (anhydrous), filtered through a fiberglass column, and then stored in a 2-mL amber vial.

The methylated FAs were analyzed with a gas chromatograph equipped with an Omegawax 320 capillary column (30 m × 0.32-m internal diameter × 0.25-μm film thickness). The oven temperature was programmed to run at 140°C for 5 min and then to increase by 4°C every 30 s until reaching 240°C. The temperature of the injector and flame ionization detector was established at 260°C. The injection volume of the gas chromatograph was 1 μL. Helium was used as the carrier gas at a flow rate of 3 mL/min. The FAs were identified by comparing the retention time of the samples with a mix of 37 FA methyl ester standards ranging from C4 to C24. Quantification was carried out using the normalization method (Method 963.22, AOAC 1998). Results are presented as percentages of the FA content.

**Analysis of amino acids.**—Amino acids (AA) bound in protein were analyzed according to the methods of Lindroth and Mopper (1979) as described below.

**Amino acid sample preparation and hydrolysis.**—Sample size depended on the protein content of the nonlipid sample: 1 mg was used if the sample contained over 40% protein, whereas 3 mg were used when the sample contained less than 40% protein. The samples were placed in hydrolysis tubes with 3 mL of 6-M HCl and were vacuum sealed for 3 min. The tubes were then placed in a dry bath for hydrolysis at 120°C for at least 24 h depending on the time of the sample and its protein content.

**Amino acid extraction.**—The hydrolyzed sample was evaporated at 65°C in a rotator and was continually washed with 3 mL of distilled water to eliminate HCl. Amino acids were recovered by adding 1 mL of sodium citrate (pH 2.2) and were maintained at 0°C until chromatographic derivatization and quantification were achieved.

**Amino acid sample derivatization.**—Sodium citrate buffer (pH 2.2) and 40 μL of the internal standard (100 μM) were added to 100 μL of the hydrolyzed sample until a volume of 1 mL was reached. In a special liquid chromatography syringe,
250 µL of the diluted solution were mixed with 250 µL of ortho-phthalaldehyde and immediately filtered (Nylon, 0.2 µm). Finally, a 10-µL quantity of the derivatization solution was injected for high-performance liquid chromatography (HPLC; Varian) at CIAD–Culiacán. The time of derivatization and sample injection into the HPLC did not exceed 2 min after processing.

Determination of amino acid profiles by reverse-phase HPLC.—Reverse-phase HPLC was carried out in an HPLC system equipped with a fluorescence detector, a 10-µL injector, and a column (C18; 5 µm × 150 mm × 4.6 mm). In the mobile phase, solvent A contained sodium acetate buffer (0.1 M; pH 7.2), methanol, and tetrahydrofuran at 9.00:0.95:0.05 by volume, and solvent B was methanol. The AAs were identified and quantified by comparing the sample retention time with known concentrations of AA standards using the computational program (Varian Star Chromatography, version 4.0).

Statistical Analyses
Copepod production variables and water characteristics were compared over time by use of Student’s t-test and the Mann–Whitney U-test (P = 0.05) after the normality of variables had been confirmed with the Lilliefors normality test and Bartlett’s homoscedasticity test. The same procedure was used to evaluate larval data by parametric and nonparametric one-way ANOVAs. Larval results were evaluated with the Kruskal–Wallis test and Student–Newman–Keuls multiple comparison test. All statistical tests were conducted using SigmaStat 3 software, with significance assessed at P < 0.05.

RESULTS
Copepod Culture
The 7,000-L tank contained a culture of pure P. euryhalinus specimens. Variation in dissolved oxygen (6.9–8.6 mg/L) was not significant. Significant variation in temperature (21.4–29.5°C) was observed; this variation did not generate differences in copepod production (6,200–9,400 copepods/L) but apparently affected the development and growth rates of the different life stages, as nauplii were more abundant at lower temperatures and adults were more abundant at 29.5°C (Table 1).

Larval Experiment
After a stocking period of 24 h, 40.20% of the Spotted Rose Snapper eggs had hatched. At 1 dph, larval survival rate did not differ significantly among treatments and averaged 61.8%. Water variables were maintained at the following levels: temperature at 27.3°C (SD = 1.5), salinity at 30.9 psu (SD = 1.3), dissolved oxygen at 8.6–6.9 mg/L, and oxygen saturation at 80.0% (SD = 7). By 15 dph, larvae that were fed P. euryhalinus exclusively (treatment 1) were significantly longer (6.6 mm, SD = 0.2) and heavier (3.04 mg, SD = 0.68) than larvae that received both copepods and rotifers (treatment 2; 4.8 mm, SD = 0.5; 1.50 mg, SD = 0.12); larvae that were given B. rotundiformis exclusively (treatment 3) were the smallest (3.8 mm, SD = 0.2; 0.93 mg, SD = 0.35; Table 2). Larval SGR was significantly higher for fish that received copepods as feed (48.4% BW/d) than for fish that were given rotifers (7.3% BW/d). Larval wet weight already differed significantly among treatments at 6 dph, with mean weights of 0.06 mg (SD = 0.02) for treatment 1; 0.03 mg (SD = 0.01) for treatment 2; and 0.03 mg (SD = 0.1) for treatment 3. Survival at 15 dph was significantly higher for larvae that were fed only rotifers than for larvae in the other two treatments ([treatment 3: 5.2%] > [treatment 2: 2.2%] > [treatment 1: 1.4%]; Table 2). All larvae belonging to treatments 1 and 2 were returned to their culture tanks after the measurement procedures, whereas larvae from treatment 3 died during the procedures.

Biochemical Analyses
Copepods had higher protein and lipid content (Table 3) and a higher AA protein-bound content (Table 4) than rotifers; copepods also contained three times more lysine and twice the amount of methionine (Table 4). Copepods had higher levels

![Table 1](https://example.com/table1.png)

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Density (copepods/L)</th>
<th>Life stage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21.4 ± 1.4 z</td>
<td>6,200 ± 2,850</td>
<td>Nauplii: 44.8 ± 4.4 y</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Copepodites: 20.3 ± 3.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adults: 35.3 ± 6.7 w</td>
</tr>
<tr>
<td>25.4 ± 0.8 y</td>
<td>7,200 ± 3,500</td>
<td>Nauplii: 48.2 ± 23.7 y</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Copepodites: 18.8 ± 7.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adults: 33.9 ± 16.0 w</td>
</tr>
<tr>
<td>29.5 ± 0.7 x</td>
<td>9,400 ± 2,200</td>
<td>Nauplii: 35.5 ± 22.8 z</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Copepods: 24.2 ± 4.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adults: 40.3 ± 20.6 v</td>
</tr>
</tbody>
</table>

![Table 2](https://example.com/table2.png)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment 1</th>
<th>Treatment 2</th>
<th>Treatment 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>TL (mm)</td>
<td>6.60 ± 0.20 z</td>
<td>4.80 ± 0.50 y</td>
<td>3.80 ± 0.20 x</td>
</tr>
<tr>
<td>Wet weight (mg)</td>
<td>3.04 ± 0.68 z</td>
<td>1.50 ± 0.12 y</td>
<td>0.93 ± 0.35 x</td>
</tr>
<tr>
<td>Survival (%)</td>
<td>1.4 x</td>
<td>2.2 y</td>
<td>5.2 z</td>
</tr>
</tbody>
</table>

![Table 3](https://example.com/table3.png)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment 1</th>
<th>Treatment 2</th>
<th>Treatment 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (mg/g)</td>
<td>1.40 ± 0.10 y</td>
<td>1.20 ± 0.05 z</td>
<td>1.30 ± 0.05 x</td>
</tr>
<tr>
<td>Lipid (mg/g)</td>
<td>0.90 ± 0.05 z</td>
<td>0.80 ± 0.05 y</td>
<td>1.00 ± 0.05 x</td>
</tr>
</tbody>
</table>

![Table 4](https://example.com/table4.png)

<table>
<thead>
<tr>
<th>AA</th>
<th>Treatment 1</th>
<th>Treatment 2</th>
<th>Treatment 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine (mg/g)</td>
<td>0.30 ± 0.05 x</td>
<td>0.20 ± 0.05 y</td>
<td>0.35 ± 0.05 z</td>
</tr>
<tr>
<td>Methionine (mg/g)</td>
<td>0.10 ± 0.05 z</td>
<td>0.05 ± 0.05 x</td>
<td>0.15 ± 0.05 y</td>
</tr>
</tbody>
</table>
TABLE 3. Proximate analyses (dry matter basis; mean ± SD) of live-feed organisms (copepods Pseudodiaptomus euryhalinus and rotifers Brachionus rotundiformis) and of Spotted Rose Snapper larvae that received one of three live-feed treatments from 3 to 15 d posthatch: (1) P. euryhalinus only (1,000 copepods/L); (2) a mix of P. euryhalinus (500 copepods/L) and B. rotundiformis (5,000 rotifers/L); and (3) B. rotundiformis only (10,000 rotifers/L). Within a given row, means with different letters are significantly different (P < 0.05; z > y; x > w).

<table>
<thead>
<tr>
<th>Variable</th>
<th>P. euryhalinus</th>
<th>B. rotundiformis</th>
<th>Treatment 1</th>
<th>Treatment 2</th>
<th>Treatment 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (%)</td>
<td>89.1 ± 0.3 z</td>
<td>79.0 ± 2.4 y</td>
<td>79.1 ± 0.1 w</td>
<td>80.03 ± 0.5 xw</td>
<td>81.0 ± 0.1 x</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>5.0 ± 0.2 y</td>
<td>5.7 ± 0.0 z</td>
<td>15.9 ± 0.1 w</td>
<td>16.1 ± 0.0 x</td>
<td>16.7 ± 0.1 x</td>
</tr>
<tr>
<td>Lipid (%)</td>
<td>10.6 ± 0.1 z</td>
<td>9.4 ± 0.1 y</td>
<td>12.7 ± 0.1 w</td>
<td>11.1 ± 0.1 w</td>
<td>9.1 ± 0.1 x</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>73.7 ± 0.1 z</td>
<td>63.3 ± 0.0 y</td>
<td>63.5 ± 0.11 wx</td>
<td>64.3 ± 0.1 x</td>
<td>67.2 ± 0.1 x</td>
</tr>
</tbody>
</table>

of the FAs arachidonic acid (ARA) and docosahexaenoic acid (DHA) and slightly lower levels of eicosapentaenoic acid (EPA) than rotifers. Larvae that were fed only rotifers (treatment 3) had the highest protein content, while larvae that were given only copepods (treatment 1) had the lowest protein content; these levels were statistically different from those of larvae that received the mixed diet of copepods and rotifers (treatment 2). In contrast, the highest lipid content was observed in larvae from treatment 1, and the lowest lipid level was found in larvae from treatment 3 (Table 3). Total AAs and methionine were highest in larvae that were fed only copepods and lowest in larvae that received only rotifers (Table 4). Lysine content was highest in larvae that were given copepods exclusively and lowest in larvae that received the mixed diet (Table 4). For the essential FAs, there was no clear pattern: total FAs and EPA were highest in larvae that were fed the copepod-only diet and lowest in those that received the rotifer-only diet, whereas DHA and ARA were highest in larvae that were given only copepods and lowest in larvae that were fed the mixed diet (Table 5).

DISCUSSION

Copepod Culture

The production of P. euryhalinus under continuous-culture technology demonstrates that copepods are a feasible source

TABLE 4. Amino acid content (% of dry protein content) of live feed (copepods Pseudodiaptomus euryhalinus and rotifers Brachionus rotundiformis) and of Spotted Rose Snapper larvae that received one of three live-feed treatments from 3 to 15 d posthatch: (1) P. euryhalinus only (1,000 copepods/L); (2) a mix of P. euryhalinus (500 copepods/L) and B. rotundiformis (5,000 rotifers/L); and (3) B. rotundiformis only (10,000 rotifers/L). Within a given row, means with different letters are significantly different (P < 0.05; z > y; x > w; v).

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>P. euryhalinus</th>
<th>B. rotundiformis</th>
<th>Treatment 1</th>
<th>Treatment 2</th>
<th>Treatment 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Essential amino acids (EAAs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leucine</td>
<td>0.00</td>
<td>2.66</td>
<td>0.00</td>
<td>3.21</td>
<td>2.97</td>
</tr>
<tr>
<td>Valine</td>
<td>0.95</td>
<td>0.75</td>
<td>0.96</td>
<td>2.21</td>
<td>1.70</td>
</tr>
<tr>
<td>Lysine</td>
<td>5.69</td>
<td>1.39</td>
<td>2.68</td>
<td>1.05</td>
<td>2.53</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>3.56</td>
<td>2.24</td>
<td>4.75</td>
<td>2.28</td>
<td>1.11</td>
</tr>
<tr>
<td>Arginine</td>
<td>2.91</td>
<td>1.52</td>
<td>2.94</td>
<td>7.76</td>
<td>1.42</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>0.00</td>
<td>0.93</td>
<td>0.29</td>
<td>0.63</td>
<td>0.58</td>
</tr>
<tr>
<td>Methionine</td>
<td>3.27</td>
<td>1.58</td>
<td>3.37</td>
<td>1.35</td>
<td>0.63</td>
</tr>
<tr>
<td>Total EAAs</td>
<td>16.38</td>
<td>11.07</td>
<td>14.99</td>
<td>18.49</td>
<td>10.94</td>
</tr>
<tr>
<td>Nonessential amino acids (NEAAs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>11.75</td>
<td>8.61</td>
<td>15.62</td>
<td>11.62</td>
<td>10.68</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>8.74</td>
<td>7.28</td>
<td>11.23</td>
<td>7.81</td>
<td>8.70</td>
</tr>
<tr>
<td>Alanine</td>
<td>4.10</td>
<td>1.82</td>
<td>4.46</td>
<td>3.87</td>
<td>3.28</td>
</tr>
<tr>
<td>Glycine–threonine</td>
<td>8.50</td>
<td>3.94</td>
<td>9.55</td>
<td>8.07</td>
<td>7.27</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>1.68</td>
<td>1.04</td>
<td>1.38</td>
<td>1.05</td>
<td>0.75</td>
</tr>
<tr>
<td>Total NEAAs</td>
<td>41.96</td>
<td>29.31</td>
<td>50.48</td>
<td>37.78</td>
<td>37.10</td>
</tr>
<tr>
<td>Total EAAs + NEAAs</td>
<td>58.34 z</td>
<td>40.38 y</td>
<td>65.47 x</td>
<td>57.27 w</td>
<td>48.04 v</td>
</tr>
</tbody>
</table>
TABLE 5. Fatty acid content (% of total lipids) of live feed (copepods *Pseudodiaptomus euryhalinus* and rotifers *Brachionus rotundiformis*) and of Spotted Rose Snapper larvae that received one of three live-feed treatments from 3 to 15 d posthatch: (1) *P. euryhalinus* only (1,000 copepods/L); (2) a mix of *P. euryhalinus* (500 copepods/L) and *B. rotundiformis* (5,000 rotifers/L); and (3) *B. rotundiformis* only (10,000 rotifers/L). Within a given row, means with different letters are significantly different (one-way ANOVA: *P* < 0.05; *z* > *y*; *x* > *w* > *v*).

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th><em>P. euryhalinus</em></th>
<th><em>B. rotundiformis</em></th>
<th>Treatment 1</th>
<th>Treatment 2</th>
<th>Treatment 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>MYRISTIC (14:0)</td>
<td>0.76</td>
<td>0.24</td>
<td>0.29</td>
<td>0.33</td>
<td>0.14</td>
</tr>
<tr>
<td>PENTADECANOIC (15:0)</td>
<td>0.06</td>
<td>0.08</td>
<td>0.05</td>
<td>0.08</td>
<td>0.04</td>
</tr>
<tr>
<td>PALMITIC (16:0)</td>
<td>2.37</td>
<td>1.27</td>
<td>2.47</td>
<td>2.89</td>
<td>1.96</td>
</tr>
<tr>
<td>PALMITOLEIC (16:1, cis-9)</td>
<td>1.56</td>
<td>1.24</td>
<td>0.54</td>
<td>0.57</td>
<td>0.35</td>
</tr>
<tr>
<td>HEPTADECANOIC (17:0)</td>
<td>0.09</td>
<td>0.05</td>
<td>0.04</td>
<td>0.04</td>
<td>0.14</td>
</tr>
<tr>
<td>cis-10-HEPTADECENOIC (17:1)</td>
<td>0.10</td>
<td>0.06</td>
<td>0.10</td>
<td>0.13</td>
<td>0.08</td>
</tr>
<tr>
<td>STEARIC (18:0)</td>
<td>1.05</td>
<td>2.66</td>
<td>3.33</td>
<td>3.29</td>
<td>2.78</td>
</tr>
<tr>
<td>OLEIC (18:1[n-9])</td>
<td>0.33</td>
<td>0.67</td>
<td>1.33</td>
<td>1.24</td>
<td>0.93</td>
</tr>
<tr>
<td>LINOLEIC (18:2[n-6])</td>
<td>0.09</td>
<td>0.02</td>
<td>0.002</td>
<td>0.00</td>
<td>0.05</td>
</tr>
<tr>
<td>α-LINOLENIC (18:3[n-3])</td>
<td>0.26</td>
<td>0.06</td>
<td>0.30</td>
<td>0.37</td>
<td>0.64</td>
</tr>
<tr>
<td>18:3</td>
<td>0.71</td>
<td>0.03</td>
<td>0.15</td>
<td>0.14</td>
<td>0.13</td>
</tr>
<tr>
<td>EICOSENOIC (20:1)</td>
<td>0.02</td>
<td>0.44</td>
<td>0.56</td>
<td>0.44</td>
<td>0.18</td>
</tr>
<tr>
<td>EICOSADENOIC (20:2)</td>
<td>0.03</td>
<td>0.05</td>
<td>0.09</td>
<td>0.07</td>
<td>0.11</td>
</tr>
<tr>
<td>EICOSATRISOIC (20:3)</td>
<td>0.24</td>
<td>0.27</td>
<td>0.15</td>
<td>0.08</td>
<td>0.02</td>
</tr>
<tr>
<td>20:3</td>
<td>0.01</td>
<td>0.12</td>
<td>0.09</td>
<td>0.04</td>
<td>0.20</td>
</tr>
<tr>
<td>ARACHIDONIC (20:4[n-6])</td>
<td>1.35</td>
<td>0.89</td>
<td>0.57</td>
<td>0.24</td>
<td>0.34</td>
</tr>
<tr>
<td>EICOSAPENTAOIC (20:5[n-3])</td>
<td>0.16</td>
<td>0.19</td>
<td>0.68</td>
<td>0.52</td>
<td>0.20</td>
</tr>
<tr>
<td>DOCOSAHEXAENOIC (22:6[n-3])</td>
<td>1.38</td>
<td>0.75</td>
<td>1.47</td>
<td>0.44</td>
<td>0.73</td>
</tr>
<tr>
<td>22:2</td>
<td>0.03</td>
<td>0.19</td>
<td>0.24</td>
<td>0.07</td>
<td>0.04</td>
</tr>
<tr>
<td>BEHENIC (22:0)</td>
<td>0.00</td>
<td>0.08</td>
<td>0.21</td>
<td>0.10</td>
<td>0.04</td>
</tr>
<tr>
<td>Total fatty acids</td>
<td>10.60 z</td>
<td>9.36 y</td>
<td>12.66 x</td>
<td>11.10 w</td>
<td>9.09 v</td>
</tr>
</tbody>
</table>

of live prey for Spotted Rose Snapper larviculture, even under temperature variation (21.4–29.5°C), and can be used as first feed for larvae during the spawning season. The copepod culture system was easy to maintain and provided sufficient production with minimum labor and expense. Because wild copepods may introduce pathogens into the larval culture system (Puello-Cruz and González-Rodríguez 2011) and are competitors and predators of larvae (Ajiboye et al. 2011), controlled or semi-controlled cultures of copepods are preferable. Furthermore, fluctuation in natural supplies of zooplankton can lead to sudden declines in wild copepod production, thereby affecting larviculture (Tønnessen Busch et al. 2010); when copepods are cultured, as in the present study, the supply is likely to be more constant (van der Meeren et al. 2014). Although the nutritional content of copepods can vary with development stage and environmental conditions (van der Meeren et al. 2008), *P. euryhalinus* in the present study had higher total protein, lipid, ARA, DHA, and AA content than *B. rotundiformis*. Because the experiment was conducted outdoors, the organisms endured different temperatures. The annual temperature in Mazatlán fluctuates from 11.5°C in the coldest months (December–March) to 36.2°C in the hottest months (June–September; CONAGUA 2011). Temperature variations affect the developmental stages of copepods rather than productivity; it appears that low temperatures enhance the nauplius stages, whereas high temperatures improve the adult stages (Table 1). Temperature is an important factor for copepod population growth and egg hatching rates (Rhyne et al. 2009).

**Larval Experiment**

The superiority of the copepod diet in the present study, in terms of producing greater lengths and wet weights of the Spotted Rose Snapper larvae, supports the results of other studies on the use of copepods for larval feeding. For example, copepods *Acartia tonsa* and rotifers were used as a first feed for Southern Flounder *Paralichthys lethostigma* (Wilcox et al. 2006), and copepods and rotifers were fed to Silver Seabream *Pagrus auratus* (Payne et al. 2001). Copepods were also included in diets for Atlantic Cod *Gadus morhua* (Imsland et al. 2006; Koedijk et al. 2010; Tønnessen Busch et al. 2010).

The higher survival rate for Spotted Rose Snapper larvae that were fed only rotifers was similarly observed by Koedijk et al. (2010) for Atlantic Cod. Koedijk et al. (2010) attributed the higher survival to differences in prey size. The poor survival of
larvae that were fed copepods in the present study was likely related to the stocking of *P. euryhalinus* adult pairs, with the males (1,234 µm) grasping the females (1,520 µm), rather than stocking nauplii (70 µm; Puello-Cruz et al. 2013). The size and semi-benthic behavior of *P. euryhalinus* adults would make them less available than rotifers. Larval preference for copepods at the nauplius stage during the time of first feeding was also noted by van der Meeran (1991), Støttrup (2003), and Wilcox et al. (2006), with the use of nauplii as feed being associated with better survival rates, development, and growth of larvae. A low density of nauplii explains a substantial part of the low survival in our study. It may also be useful to increase the copepod supply to 4,000 nauplii/L, thus achieving greater food availability in comparison with rotifers (Lee et al. 2003). Additional studies will be required to determine the exact age at which larvae are able to capture the adult stages of copepods (Puello-Cruz et al. 2008).

Duray et al. (1996), García-Ortega et al. (2005), Abdo de la Parra et al. (2010), and Alvarez-Lajonchère et al. (2012) all concluded that the growth of larval snapper fed only rotifers was slower than the growth of larvae receiving combined diets in which copepods or *Artemia* were included.

Spotted Rose Snapper that were given *P. euryhalinus* at first feeding achieved early weaning, and from 14 dph the larvae accepted unshelled *Artemia* cysts and artificial diets. In contrast, Abdo-de la Parra et al. (2010) reported that the weaning of Spotted Rose Snapper did not take place until 30 dph. Tønnessen Busch et al. (2010) also obtained higher growth rates in Atlantic Cod when copepods were included at first feeding, and the 25-dph weaning period decreased to 16 dph.

Spotted Rose Snapper larvae that received a diet of *P. euryhalinus* exhibited increased resistance to stress, as they survived measurement procedures (length and wet weight), whereas larvae that were fed only rotifers did not. For Black Sea bass *Centropristis striata*, a dietary supplementation containing between 6% and 12% *ARA* in combination with adequate DHA : EPA ratios (≥2:1) was shown to improve stress resistance (Carrier et al. 2011). In the present study, the *ARA* concentration and DHA : EPA ratio in *P. euryhalinus* were approximately twice those in *B. rotundiformis* (Table 5); these FAs are considered the most important for larval development.

Watanabe (1988) suggested that when protein in a diet is low and lipid content is high, larvae develop a strategy of using lipids as an energy source to decrease protein oxidation and reduce protein consumption. This may explain why the protein content of larvae at the end of our experiment was highest in those that were given the rotifer-only diet and lowest in those that received the copepod-only diet, even though the rotifers had a lower protein content than the copepods.

Many species grow extremely fast during the larval period; some authors have reported the demand for high amounts of essential AAs and other micronutrients and macronutrients to produce tissue protein during ontogenetic changes, and they require energy for metabolic processes (Rønnestad et al. 1999; Civera-Cerecedo et al. 2004). Rotifers in our study presented a lower AA profile than the copepods, with less than half the amount of lysine and half the methionine—the two main AAs required during larval development (Rønnestad et al. 1999; Rajkumar and Kumoraguru Vasagam 2006). This may explain why larvae that were given exclusively rotifers achieved the lowest observed weight and smallest observed size. Polyunsaturates and phospholipids are key elements of cell membranes and may therefore also explain the differences in growth. Furthermore, taurine, which was not analyzed in the present study but is more abundant in copepods, may have a profound impact on larval growth (Mæhre et al. 2013; Hawkyard et al. 2014).

Survival and growth are directly related to the presence of essential FAs (Payne et al. 2001; McKinnon et al. 2003). The most studied FA is DHA; it is considered the most important FA for improving larval development, followed by EPA and ARA, which act as growth precursors during larval development (Glencross 2009; Carrier et al. 2011). Amounts of ARA and DHA were almost twice as high in *P. euryhalinus* as in *B. rotundiformis*, whereas EPA was similar in the two prey species. Larvae that were given only copepods had the highest values of total essential FA content, including DHA, ARA, and EPA. Gasparin and Duray (2001) found that Milkfish *Chanos chanos* larvae that were fed copepods had higher amounts of DHA than larvae that were fed rotifers and *Artemia* under laboratory conditions. Docosahexaenoic acid and EPA are related to successful growth, and ARA is related to high survival rates (Salih et al. 1995; Zheng et al. 1996). The results indicate the importance of further studies of larval stress resistance, as the larvae that were fed only rotifers exhibited the highest survival but did not tolerate handling related to measurement. After hatching, rotifers measure 100–200 µm (Boza-Abarca et al. 2008), *P. euryhalinus* nauplii measure 70 µm (SD = 0.5; Velasco-Blanco et al. 2011), and the mouth gape of each Spotted Rose Snapper larva is around 180 µm; hence, copepod nauplii would be a better option in terms of prey size. It appears also that in order to improve the culture of Spotted Rose Snapper, the amount of mixed diet (copepods plus rotifers) administered should be greater than the feed amounts established in earlier trials at CIAD. According to our results, the proposed copepod culture system can produce a harvest of between 6,200 and 9,400 copepods L⁻¹ d⁻¹ during the 5 months corresponding to the spawning period of Spotted Rose Snapper (May–September). We support the view of Støttrup (2000) that including even a limited amount of copepods in the diet will improve fish larval development, without having to eliminate rotifers as a live feed.

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