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ARTICLE

Effects of Prey Size, Microalgal Density, and Light Intensity on Survival of Pacific Red Snapper Larvae

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Abstract

Development of Pacific Red Snapper *Lutjanus peru* culture has been impeded by low survival in larval culture. We studied the effects of different culture conditions on larval survival in four experiments that examined (1) different-size prey at first feeding (nauplii of the copepods *Parvocalanus crassirostris* and *Pseudodiaptomus euryhalinus*; rotifers *Proales similis*; and a mix of these three prey types); (2) microalgal density (3×10^5 and 1×10^6 cells/mL); (3) the presence of adult copepods *P. crassirostris* in larval culture tanks compared to daily nauplius addition (Adult and Nauplius treatments); and (4) light intensity (50, 200, 500, and 1,000 lx). In experiment 1, a strong preference was observed for copepod *P. crassirostris* nauplii compared to other prey types. Survival at 15 d posthatch (DPH) was significantly higher in the *P. crassirostris* treatment (mean ± SD = 19.5 ± 10.2%) and the Mixed diet treatment (33.4 ± 9.4%). However, strange behavior of larvae was observed, followed by drastic mortality (at 15–18 DPH) when microalgal addition to larval tanks ceased. This behavior and mortality had not been observed in experiment 2 at any of the microalgal densities supplied until 20 DPH. In experiment 3, survival was evaluated at 2, 5, 7, and 9 DPH and dropped to 50% (5 DPH) in both treatments. At 9 DPH, survival was 14.8 ± 9.5% for the Adult treatment and 3.4 ± 0.9% for the Nauplius treatment. In experiment 4, a significant difference was observed for survival at 9 DPH; survival at 1,000 lx (26.5 ± 18.4%) was significantly higher than survival at 200 lx (4.9 ± 5%) and 50 lx (0.6 ± 0.6%) but was not different from survival at 500 lx (6.6 ± 3.1%). In conclusion, *P. crassirostris* nauplii are an adequate prey for first feeding of Pacific Red Snapper. Therefore, to improve larval survival, *P. crassirostris* adults should be added to the larval culture tanks. Moreover, microalgae should be present during all larval rearing, and a light intensity of 1,000 lx should be used.

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Highly valued and well known by consumers worldwide, the Pacific Red Snapper *Lutjanus peru* is a species with great aquaculture potential. Some aspects of its biology related to rearing have been studied, including size at sexual maturity (Reyna-Trujillo 1993), growth rate in sea cages (Avilés-Quevedo et al. 1996), sexual maturity in captivity, and hormonal spawning induction (Dumas et al. 2004). However, information is limited with respect to the development and refinement of larval culture methods.

The development of reliable protocols for larval rearing has been the main bottleneck to aquaculture of Pacific Red Snapper, which is also the case with many other species in the families Lutjanidae and Serranidae because of the small mouth size of the larvae (166–188 μm; Doi et al. 1997; Hagiwara et al. 2014) and their nutritional requirements. Thus, larvae of finfish in the snapper–grouper complex do not ingest the live prey organisms that are conventionally used for the rearing of marine fish larvae at first feeding, such as rotifers. Moreover, in tropical marine fish larvae, the yolk-sac reserve is small and normally depleted within 2–3 d, which makes the transition from endogenous to exogenous feeding a critical period (Yúfera and Darias 2007). A low incidence of feeding may result in starved larvae that reach the point of no return and subsequently die (Blaxter and Hempel 1963; Lasker et al. 1970). Delaying the first feeding of larval Brown-marbled Grouper *Epinephelus fuscoguttatus* for just 6 h affected gut epithelium cells and reduced the growth of different body parts (Ching et al. 2016).

A variety of prey items has been evaluated in the search for optimal first-feeding diet for larvae with smaller mouth gapes. Ciliates *Amphorellopsis acuta* and *Euplotes* sp. were ingested at first feeding by larval Palette Surgeonfish *Paracanthurus hepatus* (Nagano et al. 2000), and larval Northern Anchovy *Engraulis mordax* were fed ciliates *Strombidium* sp. (Ohman et al. 1991). Moreover, larval Gray Large-eye Bream *Gymnocranius griseus* ingested micro- and nano-sized protozoans (flagellates, ciliates, and tintinnids), bivalve larvae, copepod nauplii, and diatoms (Nakagawa and Miyashita 2008). However, the most common natural prey of marine fish larvae are copepod nauplii (Hunter 1981). Indeed, copepod species with small nauplii have been confirmed as the best candidates for first feeding of small-mouthed marine finfish larvae (Doi et al. 1997; Toledo et al. 1999). In addition to their small size, the nutritional profiles of copepods meet larval fish requirements (Hamre et al. 2008). However, the use of copepods in commercial hatcheries is still limited, largely due to problems of establishing and maintaining high-density copepod cultures under intensive rearing conditions as well as the longer generation times of copepods compared to other live prey types, such as rotifers (Ajiboye et al. 2011; Alajmi and Zeng 2014). An interesting alternative is to culture copepods directly in larval fish rearing tanks, providing a constant supply of recently hatched nauplii to the larvae (Anil et al. 2018). Although rotifers are easy to produce at high densities, the species commonly used (e.g., *Brachionus* spp.) seem to be too large for Pacific Red Snapper larvae at first feeding (Zavala-Leal et al. 2013). However, small rotifers *Proales similis* have been reported to be a viable prey item for larvae with a small mouth gape (Wullur et al. 2009).

The feeding response of larvae—and consequently their growth and survival—can also be improved by selecting appropriate culture conditions at first feeding (Planas and Cunha 1999). Factors that have been reported to affect feeding response include light intensity (Downing and Litvak 1999; Peña et al. 2004) and water turbidity (Shaw et al. 2006). These two factors are important because marine fish larvae are primarily considered to be visual feeders (Yúfera and Darias 2007). Higher light intensity is thought to increase swimming activity and enhance the possibility of prey encounters (Puvanendran and Brown 2002). However, specific light requirements can be very different among marine larval fish species (Huse 1994). Turbidity is commonly generated in larval rearing tanks by the addition of microalgae (known as the "green-water" technique). This technique has been shown to improve the contrast between the prey and the culture tank, subsequently increasing prey capture by larvae (Cobcroft et al. 2001). The green-water method also improves larval digestive functions (Cahu et al. 1998; Lazo et al. 2000) and helps to maintain the nutritional value of prey (Reitan et al. 1997).

To obtain relevant information about the culture of larval Pacific Red Snapper, the aim of this study was to evaluate prey of different sizes and characteristics at first feeding as well as to assess variations in culture conditions, specifically microalgal density and light intensity, on larval feeding response and survival.

METHODS

Egg procurement, incubation, and larval culture protocols and conditions.— For all experiments, fertilized eggs were obtained from captive Pacific Red Snapper broodstock that spawned spontaneously under confidential temperature and photoperiod conditions at the Earth Ocean Farms hatchery located in La Paz, Baja California Sur, Mexico. Eggs were transported to the Marine Culture Experimental Unit at the Centro Interdisciplinario de Ciencias Marinas, Instituto Politécnico Nacional (IPN), La Paz. At the laboratory, to separate live and dead eggs by flotation, they were placed in a 2-L container with treated seawater (36.0 ± 0.5 psu; filtered to 5 μm, chlorinated with 5 mg/L for 24 h, and neutralized with sodium thiosulfate). Floating eggs were incubated in a flow-through,

100-L cylindroconical tank equipped with a central mesh (200-μm) column (diameter = 5 cm; height = 45 cm). Seawater exchange rate was 50 L/h, light intensity was 10 lx during the daytime, and aeration was provided. Temperature and salinity were 26.0 ± 0.5°C and 36.0 ± 0.5 psu, respectively. Four hours after hatching, three samples (100 mL) were taken to estimate larval density. Finally, larvae were distributed in the rearing experimental system at a density of 50 larvae/L. Hatching was estimated by taking three samples from the incubation tank (≈100–150 eggs/sample) that were placed into separate plastic storage bags with 2 L of treated seawater. Bags were inflated by injecting air and were maintained in a water bath at 26.0 ± 0.5°C. The hatching percentage (>85%) was evaluated 24 h later.

Larval rearing was achieved in a recirculating aquaculture system using 200-L cylindroconical tanks (height = 67 m; diameter = 60 cm; n = 16) with black walls, white bottoms, and a central sieve outlet. The recirculating aquaculture system was equipped with a 245-W (0.333-hp) water pump (RK2 Systems); a 1,200-L reservoir; a high-rate silica sand filter (Triton II; PAC-FAB); a degassing column packed with plastic bio-balls; a 25-W ultraviolet lamp (Emperor Aquatics); and a fluidized sand biofilter (Seastorm). Each tank was provided with two 28-W fluorescent lamps (Philips). Light intensity was regulated by adding shade cloths and adjusting the distance between lamps and the water surface. Light intensity was measured at the water surface with a photometer (L246373; Extech Instruments). Sunrise and sunset were simulated using a programmable switch connected to an extra lamp (50 lx). Dissolved oxygen, salinity, and temperature were measured daily with a smarTROLL multiparameter handheld meter (In-Situ). Total ammonia was determined using a commercial colorimetric kit (API Marine). Physicochemical rearing conditions for all experiments are described in Table 1.

During experiments 1 and 2, the prey supply started at 3 d posthatch (DPH) considering that Zavala-Leal et al. (2015) reported fully pigmented eyes, an open mouth and anus, and an articulated jaw in Pacific Red Snapper larvae at 72 h posthatch. For experiments 3 and 4, however, the prey supply started at 2 DPH to ensure that the early developing larvae would have food when they were ready to eat.

Live-feed production and body width measurement.— Seawater used for all live-feed production was treated as described previously. Rotifers *P. similis* were fed with *Nannochloropsis oculata* (9×10^6 cells/mL). No additional enrichment was used. In experiment 2, rotifers *Brachionus rotundiformis* were cultured using a commercial diet (RotiGrow-OneStep; Reed Mariculture) following the recommended procedure.

Culture of copepods *Pseudodiaptomus euryhalinus* started with 4 mature adults/mL. A mixture of microalgae was provided on the first day (*Chaetoceros calcitrans*: 9×10^5 cells/mL; *Tetraselmis suecica*: 1×10^5 cells/mL). Every 4 d, tanks were harvested. *Parvocalanus crassirostris* was cultured by adding a mixture of microalgae daily (*Isochrysis galbana*: 7×10^4 cells/mL; *C. calcitrans*: 2×10^4 cells/mL; *T. suecica*: 2×10^4 cells/mL). Every day or every 2 d, nauplii were harvested.

Brine shrimp *Artemia* sp. nauplii were obtained from commercial cysts (INVE Aquaculture Nutrition). For hatching, cysts were chlorinated and decapsulated. A portion of the newly hatched nauplii were offered to larvae. Twelve hours later, metanauplii were enriched with Selco S-Presso (INVE) for 12 h by following the recommended procedure.

Samples of prey offered at first feeding (n = 100) were fixed using a buffered formaldehyde solution (5%) and immediately photographed with a digital camera (Lumerera Infinity 1) coupled to a stereoscope (Olympus SZ40). Body width (BW) was measured later with Image-Pro Plus software (Media Cybernetics). Spines and appendages were not included in copepod nauplius measurements.

Feeding incidence and feeding intensity.— At 3 DPH, feeding incidence and intensity were determined 6 h after adding live feed to larval tanks; on subsequent days, feeding incidence and intensity were determined 2 h after the addition of live feed (Zavala-Leal et al. 2013). Larvae (n = 25–30 per treatment) were anesthetized with 2-phenoxyethanol (4%), and feeding incidence (equation 1 below) was calculated as the percentage of larvae with prey in the digestive tract. Feeding intensity (equation 2) was determined by dissecting the larvae under a binocular

TABLE 1. Physicochemical conditions (mean ± SD) in the four experiments with larval Pacific Red Snapper.

Variable	Experiment			
	1	2	3	4
Temperature (°C)	24.2 ± 0.3	25.6 ± 0.8	25.0 ± 0.2	25.7 ± 0.3
Dissolved oxygen (mg/L)	5.5 ± 1.3	5.5 ± 0.7	4.0 ± 0.5	3.6 ± 0.4
Salinity (psu)	35.0 ± 0.8	37.2 ± 0.4	34.3 ± 0.1	31.3 ± 0.9
Ammonia (NH_3/NH^{4+}; mg/L)	0.0 ± 0.8	0.2 ± 0.2	0.2 ± 0.1	0.4 ± 0.2

microscope and counting the number of prey items per larva (Yin and Blaxter 1987):

$$\text{Feeding incidence} = \frac{\text{Number of larvae with prey items}}{\text{Number of larvae in the sample}} \times 100 \quad (1)$$

$$\text{Feeding intensity} = \frac{\sum \text{Number of prey items in the digestive tract}}{\text{Number of larvae with food}}. \quad (2)$$

Ivlev's electivity index.— Ivlev's (1961) electivity index was evaluated in experiment 2 during the co-feeding period (n = 30 larvae/treatment). The index considers the average proportion of each prey type in the digestive tract and its density in the tank. Values of the index range from −1 to 1, where 1 indicates maximum selection of the prey type, 0 indicates that the prey is consumed proportionally to the number of organisms in the tank, and −1 indicates that no consumption of the prey type took place. Ivlev's index (Iv) was calculated as

$$Iv = \frac{r_i - p_i}{r_i + p_i},$$

where r_i is the percentage of prey type i in the larval digestive tract and p_i is the percentage of prey type i in the tank.

Experiment 1: effect of different prey at first feeding on survival.— Four different treatments were used, each with four replicates. Names assigned to treatments were based on the prey used at first feeding and maintained until the end of the experiment. Four feeding treatments were applied from 3 to 10 DPH: rotifers *P. similis* (treatment 1); nauplii of the copepod *P. euryhalinus* (treatment 2); nauplii of the copepod *P. crassirostris* (treatment 3); and a mixture of these three prey types in equal proportions (Mixed diet; treatment 4). In treatments 3 and 4, *P. crassirostris* copepod adults were added at 2 DPH. Prey densities were selected in accordance with those commonly used in other studies (Abdo-de la Parra et al. 2010; Cassiano et al. 2011). From 8 DPH, the number of first-feeding prey was progressively decreased and the density of *P. euryhalinus* copepodites increased each day in all tanks (8 DPH: 0.5 copepodite/mL; 9 DPH: 1 copepodite/mL; 10 DPH: 2 copepodites/mL). From 11 to 15 DPH, only *P. euryhalinus* copepodites (4 copepodites/mL) were offered to larvae. Newly hatched brine shrimp nauplii and metanauplii were offered from 16 to 24 DPH. Co-feeding with *P. euryhalinus* copepodites lasted 3 d (16–18 DPH). Enriched brine shrimp juveniles were added from 24 to 30 DPH several times per day. Prey densities are shown in Figure 1.

Larvae were sampled at 3, 4, and 5 DPH to determine feeding incidence and intensity. At 3 DPH, larvae were sampled (n = 66) to determine the mouth width (MW). They were anesthetized as previously described and were later fixed in formalin at 5%. Larvae were placed in a ventral position and photographed as already described. Using Image-Pro Plus version 4.5, the distance between jaw–maxilla joints was measured (Russo et al. 2009).

From 3 to 10 DPH, to maintain the nutritional quality of each prey type and nauplius production by *P. crassirostris*

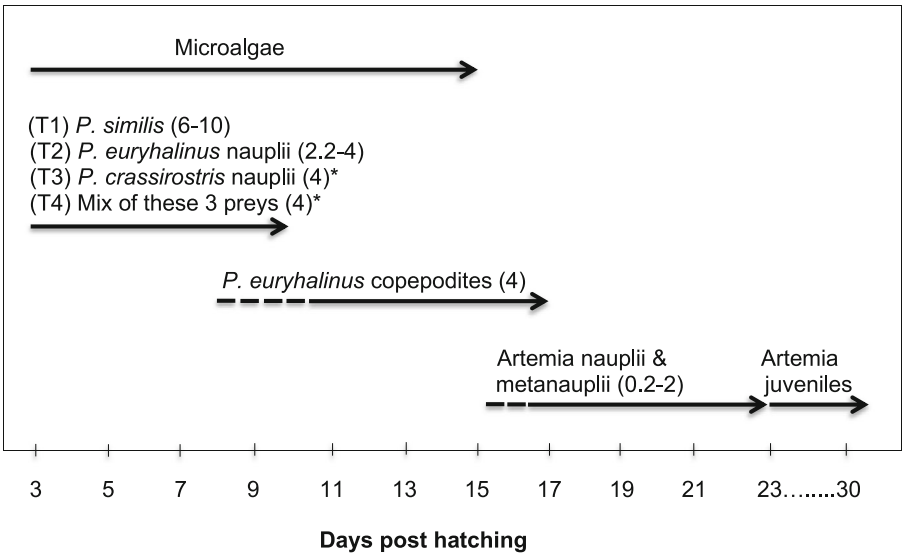

FIGURE 1. Feeding schedule for Pacific Red Snapper larvae in experiment 1. Dashed lines indicate the co-feeding period. For each treatment (T1 = *Proales similis*; T2 = *Pseudodiaptomus euryhalinus* nauplii; T3 = *Parvocalanus crassirostris* nauplii; T4 = mix of the three prey types), the numbers in parentheses refer to prey density (organisms/mL). Asterisks indicate that *P. crassirostris* adults were added at 2 d posthatch in T3 (2.0 adults/mL) and T4 (0.7 adult/mL).

adults, different microalgal species were added to each treatment, similar to those used for each specific live-feed production (Table 2). *Nannochloropsis oculata* (3×10^5 cells/mL) and *T. suecica* (7×10^3 cells/mL) were used in all tanks to achieve green-water conditions from 11 to 15 DPH. Light intensity at the water surface was 500 lx during the experiment, and a photoperiod of 12 h light : 12 h dark was applied.

From 15 to 30 DPH, tank bottoms were cleaned and dead larvae were siphoned daily and counted to evaluate survival. All dead larvae had been considered alive at 15 DPH. Sampled larvae were not considered in survival evaluations. Final survival was determined by emptying the tanks and counting the remaining larvae. Survival at 15 DPH was evaluated retrospectively by considering the number of larvae that were alive at 30 DPH and the total number of dead larvae that were observed during the daily siphoning from 16 to 30 DPH.

Experiment 2: microalgal density.— Based on the survival results obtained in experiment 1, the duration of the microalgal supply was extended and two different microalgal densities were evaluated. Nauplii of *P. crassirostris* were added to all tanks from 3 to 10 DPH. Rotifers *B. rotundiformis* were offered from 7 to 20 DPH. Co-feeding lasted 4 d (7–10 DPH). Copepod nauplii were decreased from 6 to 2 nauplii/mL, while rotifers were increased from 2 to 10 rotifers/mL. From 3 to 8 DPH, a mix of microalgae (3×10^5 cells/mL) composed of *N. oculata* (1.8×10^5 cells/mL), *I. galbana* (6×10^4 cells/mL), *T. suecica* (3×10^4 cells/mL), and *C. calcitran*s (3×10^4 cells/mL) was added to the rearing tanks. From 9 to 20 DPH, two microalgal densities with four replicates were applied: *N. oculata* at 3×10^5 cells/mL (Low microalgal density) were added to four tanks; and *N. oculata* at 1×10^6 cells/mL (High microalgal density) were added to the four remaining tanks. Light intensity was 500 lx during the experiment, with a photoperiod of 12 h light : 12 h dark. Nocturnal water exchange (100% tank volume) was initiated at 10 DPH as well as the removal of detritus and collection of dead larvae by siphoning every other day thereafter. The experiment ended at 20 DPH. Survival was evaluated from 9 to 20 DPH as described for experiment 1. Larvae were sampled at 3, 4, and 5 DPH for feeding incidence and at 10 DPH for feeding incidence and intensity. Ivlev's index was evaluated at 10 DPH.

Experiment 3: addition of adult copepods Parvocalanus crassirostris *to larval tanks to ensure the presence of nauplius stages I–II.*— The third experiment was designed to determine whether the high survival observed at 15 DPH in experiment 1 could be explained by the presence of *P. crassirostris* adults. In this experiment, two treatments were applied. Nineteen tanks were stocked with yolk-sac larvae just after hatching. At 2 DPH, *P. crassirostris* nauplii were added to eight tanks daily (4 nauplii/mL; Nauplius treatment). To maintain a constant production of *P. crassirostris* nauplius stages I and II, mature *P. crassirostris* adults were added to eight other tanks at 2 DPH (0.8 adult/mL; Adult treatment), and nauplii harvested from copepod culture were also added daily (4 nauplii/mL). Three tanks were left without live feeds and were emptied at 2 DPH to evaluate survival. A mix of microalgae (300,000 cells/mL) was added to the rearing tanks as described for experiment 2. Nocturnal water exchange (100% tank volume) was initiated at 6 DPH. Light intensity was 500 lx. The photoperiod was 24 h light at 2 DPH and was 12 h light : 12 h dark thereafter. Larvae were sampled to determine feeding incidence at 3, 4, and 5 DPH (10 larvae/tank). Survival was measured at 5 DPH (3 tanks), 7 DPH (2 tanks), and 9 DPH (3 tanks) in each treatment. Tanks were first siphoned to remove dead larvae and then emptied to count the live larvae.

Experiment 4: effect of light intensity on feeding incidence and survival.— In the final experiment, four different light intensities were evaluated (50, 200, 500, and 1,000 lx; 4 tanks per light intensity). Black curtains were installed around each tank to prevent light interference. The photoperiod was 24 h light at 2 DPH and 12 h light : 12 h dark thereafter. In all tanks, *P. crassirostris* adults were supplied at 2 DPH at a density of 0.8 adult/mL. From 2 to 9 DPH, *P. crassirostris* nauplii (3 nauplii/mL) were also added and a mix of microalgae (300,000 cells/mL) was added as described for experiment 2. Water exchange was initiated at 5 DPH. The experiment was conducted through 9 DPH. Larvae were sampled to

TABLE 2. Microalgal species (*Nannochloropsis oculata*, *Isochrysis galbana*, *Tetraselmis suecica*, and *Chaetoceros calcitrans*) and concentrations (cells/mL) used to create green-water conditions for each treatment in experiment 1 from 3 to 10 d posthatch during Pacific Red Snapper larval culture.

	Microalga species			
Treatment	*N. oculata*	*I. galbana*	*T. suecica*	*C. calcitrans*
Proales similis	5×10^5			
Pseudodiaptomus euryhalinus	2×10^5		2×10^5	1×10^5
Parvocalanus crassirostris		7×10^4	2×10^4	2×10^4
Mixed diet[a]	3×10^4	3×10^4	2×10^4	2×10^4

[a]The Mixed diet treatment was composed of *P. similis*, *P. euryhalinus*, and *P. crassirostris* in equal proportions.

determine feeding incidence at 3, 4, and 5 DPH (10 larvae/tank; 3 tanks/treatment). Survival was evaluated at 9 DPH by counting all larvae that were alive.

Statistical analyses.— Statistical analyses were performed using StatSoft Statistica version 10.0. Data were analyzed for normality with the Shapiro–Wilk test and for variance homogeneity with Levene's test. Student's *t*-test or one-way ANOVA was applied, followed by an a posteriori Tukey's test when significant differences were observed. Survival and feeding incidence percentages were arcsine–square root transformed.

RESULTS

Experiment 1: Effect of Different Prey at First Feeding

Prey body width and larval mouth width.— Significant differences were observed in BW (mean ± SD) between the different prey items at first feeding. The BW of *P. euryhalinus* nauplii (90 ± 7 μm) was significantly higher ($P < 0.05$) than that of *P. crassirostris* nauplii (43 ± 3 μm) and *P. similis* (41 ± 4 μm), whereas no significant difference ($P > 0.05$) was observed between *P. crassirostris* nauplii and *P. similis*. Larval MW (mean ± SD) was 198 ± 9 μm (180–220 μm) at first feeding (3 DPH).

Feeding incidence and feeding intensity.— *Parvocalanus crassirostris* was present in 100% of the guts of larvae examined at 3 DPH, whereas in the Mixed treatment, the feeding incidence (mean ± SD) was 69 ± 27% at 3 DPH and reached 97 ± 3% at 6 DPH. In contrast, the feeding incidence at 3 DPH was 17 ± 5% with *P. euryhalinus* and 2 ± 5% with *P. similis*. In these two treatments, feeding incidence reached 98–100% at 6 DPH (Figure 2A).

At 3 DPH, larvae that were fed *P. crassirostris* nauplii showed a significantly higher prey number consumed (mean ± SD = 4.3 ± 4.3 prey/larva; $P = 0.01$) compared to the other treatments. Larval consumption of the Mixed diet (3.2 ± 1.6 prey/larva) was significantly higher than the consumption of *P. similis* (1 ± 0 prey/larva; $P = 0.008$) and *P. euryhalinus* (2.1 ± 0.6 prey/larva; $P = 0.03$; Figure 2B). The same pattern was observed at 4 DPH. Larvae offered the Mixed diet on the first feeding day only consumed the *P. crassirostris* nauplii. At 5 DPH, the Mixed treatment resulted in a significantly higher feeding intensity ($P = 0.0001$) than *P. similis* or *P. euryhalinus* fed alone, whereas feeding intensity at 6 DPH was significantly higher for *P. similis* than for the two other treatments (Mixed diet and *P. euryhalinus*; $P = 0.002$).

Survival.— From 15 to 19 DPH, survival was significantly lower ($P = 0.001$) in both the *P. similis* treatment (mean ± SD = 4.3 ± 1.4%) and the *P. euryhalinus* treatment (2.2 ± 0.5%) compared to the *P. crassirostris* (19.5 ± 10.2%) and Mixed (33.4 ± 9.4%) treatments (Figure 3). Mortality of larvae was observed in the *P. crassirostris*

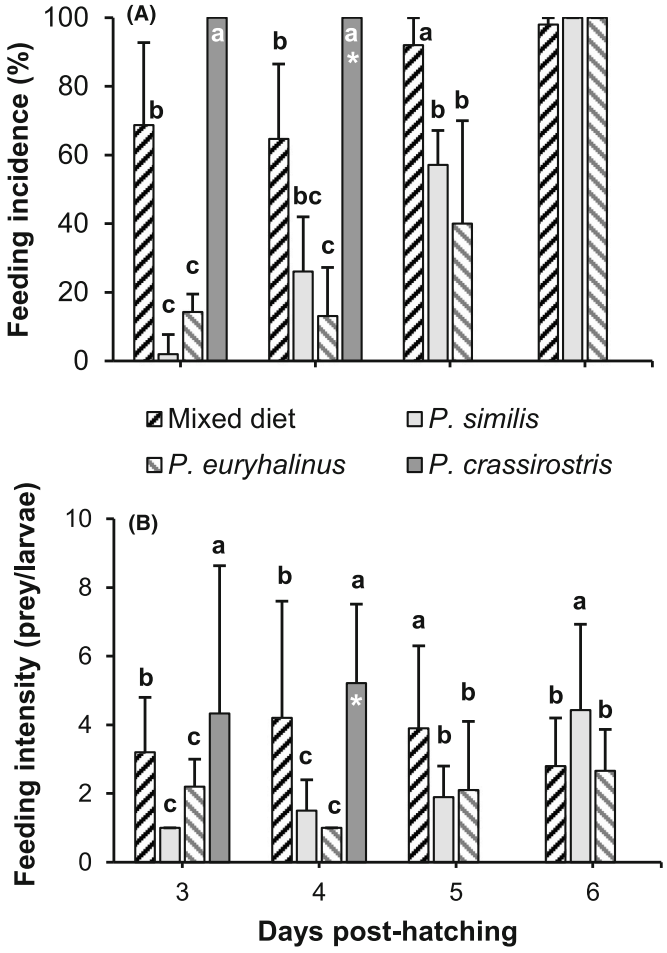

FIGURE 2. **(A)** Feeding incidence (%; mean + SD) and **(B)** feeding intensity (prey/larva; mean + SD) from 3 to 6 d posthatch for Pacific Red Snapper larvae that were offered different prey types in experiment 1 (see Figure 1). The asterisk indicates the last day of evaluation for the *P. crassirostris* treatment, as 100% feeding incidence was reached. For a given day, different letters indicate significant differences ($P < 0.05$).

and Mixed treatments (3–50% daily) from 15 to 18 DPH. After 15 DPH, microalgae were no longer added to the tanks and water was clear in the morning. Then, when the light was turned on in the morning, erratic behavior of the larvae was observed. They were sinking, hitting against the tank bottom, and dying. Moreover, dead larvae were observed daily until the end of the experiment (0.4–5.0%). No survival was observed after 20 DPH for larvae that were fed *P. euryhalinus* and after 25 DPH for larvae that were offered *P. similis*. No significant difference ($P = 0.40$) in final survival was observed between larvae that were given *P. crassirostris* (1.1 ± 0.4%) and those that were fed the Mixed diet (0.8 ± 0.4%).

Experiment 2: Microalgal Density

Feeding incidence and feeding intensity.— Feeding incidence in experiment 2 was 36.7% at 3 DPH and reached

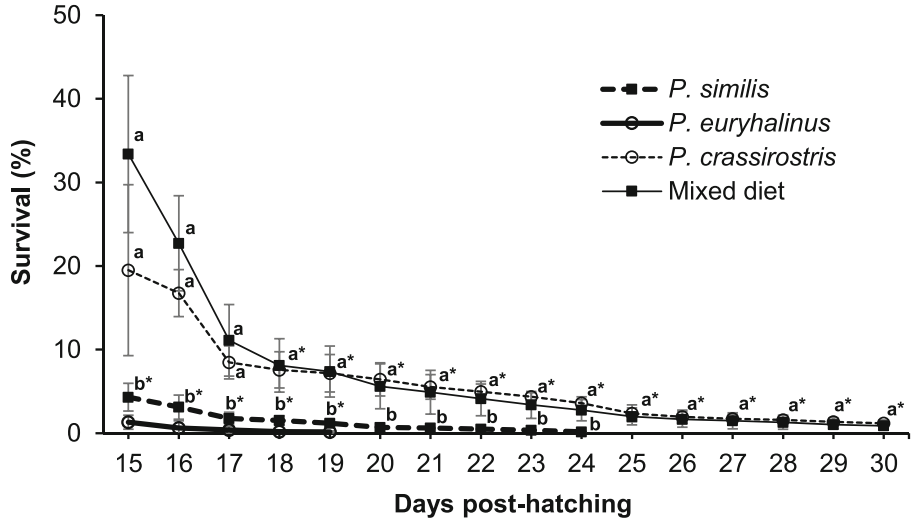

FIGURE 3. Survival (%; mean ± SD) of Pacific Red Snapper larvae from 15 to 30 d posthatch in experiment 1 (see Figure 1). Different letters indicate significant differences ($P < 0.05$; a* = no significant difference between the Mixed diet and *P. crassirostris* treatments; b* = no significant difference between the *P. similis* and *P. euryhalinus* treatments).

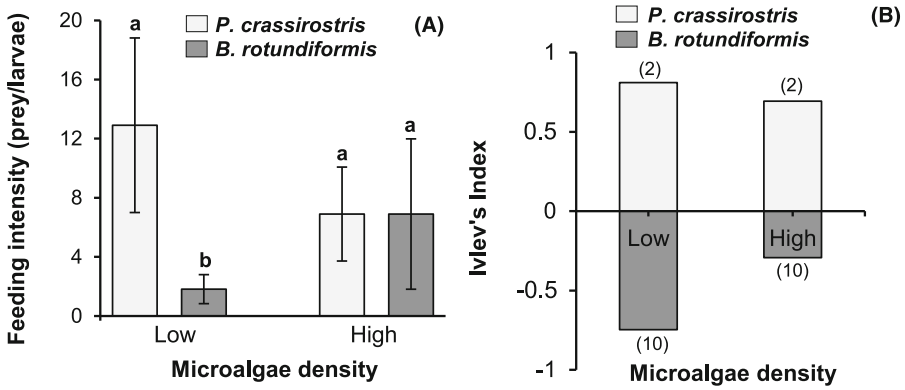

FIGURE 4. **(A)** Feeding intensity (prey/larva; mean ± SD) of Pacific Red Snapper larvae by prey type (*Parvocalanus crassirostris* and *Brachionus rotundiformis*) at 10 d posthatch (n = 30 larvae/treatment) in experiment 2. Different letters indicate significant differences ($P < 0.05$). **(B)** Ivlev's electivity index at 10 d posthatch is compared between *P. crassirostris* and *B. rotundiformis* in the Low and High microalgal density treatments. Numbers in parentheses represent the prey density (organisms/mL) supplied daily.

100% at 5 DPH. At 10 DPH, when the microalgal density treatments were initiated, no significant differences ($P = 0.90$) were observed between the number of *P. crassirostris* nauplii ingested and the number of rotifers *B. rotundiformis* ingested by larvae in the High microalgal density (1×10^6 cells/mL) treatment, while the number of *P. crassirostris* nauplii consumed was higher than the number of rotifers consumed by larvae in the Low microalgal density (3×10^5 cells/mL) treatment (Figure 4A). Positive values of Ivlev's index for larvae that received *P. crassirostris* were observed in the Low and High microalgal density treatments, whereas negative values were observed for larvae that were offered *B. rotundiformis* (Figure 4B).

Survival.— Larval survival percentages were similar between the High and Low microalgal densities during the experiment ($P > 0.05$; Figure 5). Survival (mean ± SD) at 20 DPH ranged from 0.9 ± 1.3% (Low density) to 2.5 ± 0.9% (High density; $P = 0.08$).

Experiment 3: Addition of Adult Copepods *Parvocalanus crassirostris* to Larval Tanks to Ensure the Presence of Nauplius Stages I–II

Feeding incidence.— Feeding incidence in experiment 3 increased daily from the beginning of exogenous feeding at 3 DPH until reaching 100% at 5 DPH. No significant difference (3 DPH: $P = 0.70$; 4 DPH: $P = 0.09$) was observed between the copepod Nauplius and Adult treatments.

Survival.— Survival was 90% at 2 DPH but decreased to approximately 50% at 5 DPH in both treatments.

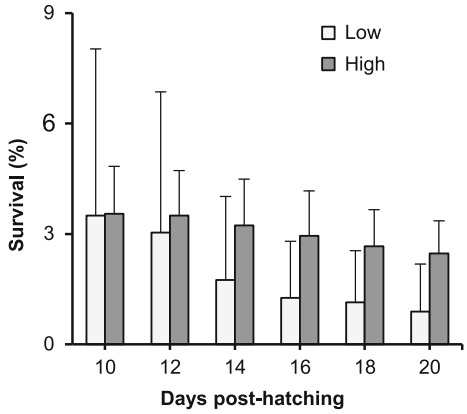

FIGURE 5. Survival (%; mean + SD) of Pacific Red Snapper larvae from 10 to 20 d posthatch in experiment 2. No significant differences were detected between Low and High microalgal density treatments on any day ($P > 0.05$).

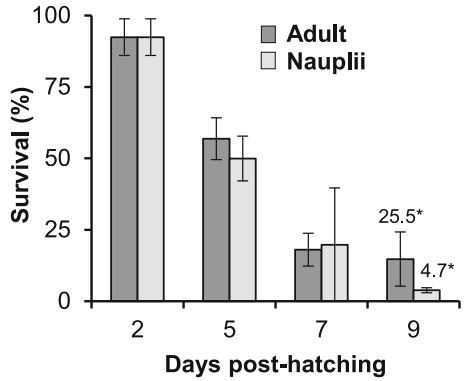

FIGURE 6. Survival (%; mean ± SD) of Pacific Red Snapper larvae in copepod *Parvocalanus crassirostris* Adult and Nauplius treatments from 2 to 9 d posthatch in experiment 3. Asterisks indicate the highest value obtained in one replicate within each treatment.

Survival did not significantly differ between copepod Nauplius and Adult treatments for 5 DPH: $P = 0.30$ and 7 DPH: $P = 0.90$. However, at 9 DPH, larvae in the Adult treatment had a higher survival percentage (mean ± SD = 14.8 ± 9.5%) than larvae in the Nauplius treatment (3.4 ± 0.9%), but the high variability among replicates did not allow for the detection of a significant difference between treatments, although close to being significant ($P = 0.07$; Figure 6). In fact, survival as high as 25.5% was observed in one Adult treatment replicate, whereas the highest survival observed in any Nauplius treatment replicate was 4.7%.

Experiment 4: Effect of Light Intensity on Feeding Incidence and Survival

Feeding incidence.— At 3 DPH during experiment 4, a significantly ($P = 0.007$) higher feeding incidence was recorded in the 500- and 1,000-lx treatments (mean ± SD = 91.7 ± 1.5% and 90.9 ± 9.1%, respectively) compared with the feeding incidence in the 50- and 200-lx treatments (57.8 ± 4.4% and 56.0 ± 21.5%, respectively). However, no significant differences were observed at 4 DPH ($P = 0.50$) or 5 DPH ($P = 0.07$). Feeding incidence was 95–100% in all treatments at 5 DPH (Figure 7A).

Survival.— A survival percentage (mean ± SD) of 26.5 ± 18.4% was observed with a light intensity of 1,000 lx, which was close to being significant ($P = 0.08$) compared to the 500-lx treatment (6.6 ± 3.1%). However, survival was as high as 48.2% and 35.3% in two replicates at 1,000 lx. Significant differences in survival were observed between the highest light intensity (1,000 lx) and the lowest intensities: 200 lx (4.9 ± 5.0%; $P = 0.02$) and 50 lx (0.6 ± 0.6%; $P = 0.002$; Figure 7B).

DISCUSSION

First-Feeding and Co-Feeding Periods

First feeding is a particularly crucial moment for the development and survival of marine finfish larvae. According to Hunter (1981), the optimal prey size at first feeding for larvae must be close to 25% of MW. Thus, the optimal prey size for Pacific Red Snapper larvae should be approximately 50 µm at first feeding. In this study, when different prey sizes were offered at first feeding, Pacific Red Snapper larvae ingested *P. crassirostris* nauplii, which are under 50 µm and up to 22% of the Pacific Red Snapper MW compared to the larger prey (*P. euryhalinus* nauplii). At first feeding in larvae of the Mangrove Red Snapper *L. argentimaculatus*, which is another species with a small mouth gape, a preference for nauplii of the copepod *Acartia sinjiensis* (width = 50 µm) over rotifers *Brachionus* sp. was observed (Doi et al. 1997). These results suggest that feeding incidence of Pacific Red Snapper larvae at first feeding is greatly affected by prey size relative to mouth size.

Although the mouth size constraint sets the limit of the maximum prey size for consumption, it often fails to explain the actual prey size selected by fish larvae. In fact, low feeding incidence was also observed with *P. similis* rotifers, which are 21% of the MW of Pacific Red Snapper larvae. Even during early stages of development, many species have a selective feeding preference not only associated with size but also with prey types (Makrakis et al. 2008; Morote et al. 2011). The prey–predator relationship has been evaluated based on prey movement, velocity, and escapes (Buskey 2005; Arndt et al. 2016; Jackson and Lenz 2016). Some researchers argue that the slow swimming movements displayed by rotifers make them easier to catch than copepod nauplii, which are known to exhibit an avoidance sensory mechanism in

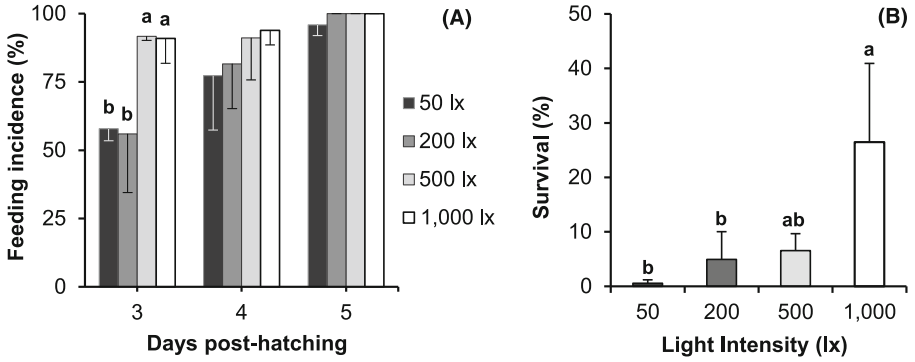

FIGURE 7. **(A)** Feeding incidence (%; mean ± SD) of Pacific Red Snapper larvae from 3 to 5 d posthatch at four different light intensities in experiment 4 and **(B)** survival (%; mean + SD) at the end of experiment 4 (9 d posthatch) for larvae in the four light intensity treatments. Different letters indicate significant differences ($P < 0.05$).

response to hydromechanical signals generated by predators (Kiørboe and Visser 1999; Strickler and Balazsi 2007; Waggett and Buskey 2008). However, in large angelfish *Pomacanthus* sp., the stop-and-go motion of copepod nauplii was required to initiate predation, contrasting with the continuous whirling motion of rotifers, which did not stimulate predatory behavior (Moe 1997). In larval Turbot *Scophthalmus maximus*, higher selection for copepod nauplii was also explained by the characteristic zigzag movement of nauplii followed by subsequent pauses for floating, which may fit the behavior of prey capture in many marine fish larvae, characterized by motionless swimming followed by S-posture and attack (van der Meeren 1991). On the other hand, the escape response in different copepod species has been studied (Buskey et al. 2017). Buskey et al. (2017) observed that the escape of *P. crassirostris* nauplii was more often in the same direction as previous swimming. If nauplii swim toward the predator, this escape response would cause them to continue approaching the larvae, possibly favoring their capture. Moreover, *P. crassirostris* nauplii swim in a "hop-and-sink"-type spiral locomotory pattern (Bradley et al. 2013). This characteristic swimming behavior could facilitate the capture of nauplii by causing them to stay closer to the larvae.

The preference of Pacific Red Snapper larvae for *P. crassirostris* nauplii at first feeding was even more evident when mixed live feeds (*P. crassirostris*, *P. euryhalinus*, and *P. similis*) were offered. In fact, only *P. crassirostris* nauplii were found in the digestive tracts on the first day of feeding. In Yellow Tang *Zebrasoma flavescens*, a preference for *P. crassirostris* nauplii was also observed when a mix of wild zooplankton was offered to larvae (Burgess and Callan 2018). The palatability of copepods relative to rotifers can also make a difference. When a mix of *P. crassirostris* nauplii and *B. rotundiformis* was offered to Mandarinfish *Synchiropus splendidus*, the copepod nauplii were never rejected once captured, whereas the rotifers were commonly rejected after capture (Shao and Zeng 2020). Those authors (Shao and Zeng 2020) suggested that copepods were substantially more palatable than rotifers.

Feeding efficiency of larval marine finfish, such as snappers, can also be affected by water turbidity. On the fourth day of co-feeding, Pacific Red Snapper larvae ingested the same numbers of rotifers and copepod nauplii in the High microalgal density treatment, while in the Low-density treatment the larvae ingested more copepod nauplii relative to rotifers. Suspended algal cells can reduce light penetration and produce a greater light intensity gradient in the water column via light attenuation (Naas et al. 1996). Marine finfish larvae are visual feeders and are highly dependent on light for prey capture and feeding success (Blaxter 1986). In the first days after hatching, the majority of marine finfish larvae exhibit a retina composed of single cones, as was already observed in Pacific Red Snapper (Zavala-Leal et al. 2015). A pure cone retina requires high light intensities for vision and color discrimination (Kjorsvik et al. 2004). The retinal rods involved in vision within a low-light environment appear later in development. In many marine fish species (Madai *Pagrus major*: Kawamura et al. 1984; Atlantic Halibut *Hippoglossus hippoglossus*: Kvenseth et al. 1996; Pacific Bluefin Tuna *Thunnus orientalis*: Kawamura et al. 2003), rods appear during or after the transformation to juveniles. The absence of rods may explain why Pacific Red Snapper larvae could not distinguish between copepod nauplii and rotifers in the High microalgal density treatment during co-feeding at 10 DPH. However, despite this difference between microalgal density treatments, Ivlev's index demonstrated that Pacific Red Snapper larvae still had preference for *P. crassirostris* nauplii at 10 DPH.

Light intensity is another factor that affects feeding efficiency of marine fish larvae. On the first feeding day in

experiment 4, a higher percentage of Pacific Red Snapper larvae were observed with prey in their digestive tracts at 500 and 1,000 lx compared to the lower light intensities (50 and 200 lx). Higher light intensity has been shown to improve the contrast of prey, allowing larval fish to effectively recognize the prey item and thus reducing the reaction time in prey capture (Puvanendran and Brown 2002). Exposure to high light intensity has also been shown to increase larval swimming and food searching activity (Batty 1987), which could result in the search of greater volumes of water. However, larval performance under particular light conditions is species-specific and can vary between very low light intensity, such as for larval Atlantic Halibut (Bolla and Holmefjord 1988), and higher light levels, such as those preferred by larval Haddock *Melanogrammus aeglefinus* (Downing and Litvak 1999). These results may be linked to larval ecology and lighting in their natural habitat (van der Meeren et al. 2007), and higher light intensity potentially could be used in the culture of Pacific Red Snapper larvae. Nevertheless, no published information is available on the photic conditions experienced by wild larvae of Pacific Red Snapper.

Survival

The onset of exogenous feeding is a crucial moment for developing fish larvae since survival can fall sharply when feeding and environmental requirements are not properly met (Yúfera and Darias 2007). High survival in fish culture is the result of a combination of various factors. Physicochemical variables, such as temperature and dissolved oxygen, must be controlled. Survival can also be impaired by a low feeding efficiency in the first days of larval culture. In the present study, experiment 3 revealed that 50% of snapper larvae died between 3 and 5 DPH during the transition from endogenous to exogenous feeding. Larvae must learn how to hunt and feed, and mortality related to the failure to complete this transition is due to what is known as the "point of no return," a point of starvation that is irreversible even if prey are ingested (May 1974). The point of no return has been reported at 5 DPH for Pacific Red Snapper, and total mortality for starved larvae occurs at 6 DPH (Peña et al. 2021).

The importance of using a small prey organism at first feeding of Pacific Red Snapper was confirmed by the significantly higher ($P = 0.001$) survival observed when *P. crassirostris* nauplii were offered at first feeding compared to *P. euryhalinus* or *P. similis*. The use of *P. crassirostris* at first feeding has also promoted higher survival in the Orange-spotted Grouper *E. coioides* (Ranjan et al. 2022) and in some ornamental fish, such as the Yellow Tang (Burgess and Callan 2018) and Marcia's Anthias *Pseudanthias marcia* (Anil et al. 2018). An advantage of copepods over rotifers is their higher levels of highly polyunsaturated fatty acids, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA; van der Meeren et al. 2008). Fish larvae are not capable of de novo biosynthesis of DHA, and DHA needs to be consumed since it is a main component of biological membranes, particularly in neural tissue (Conceição et al. 2010). The DHA : EPA ratio is also higher in copepods, with values above 2, which have been proven important for optimal development of marine fish larvae (Bell et al. 2003). Furthermore, copepods have a higher protein content and free amino acid content than either rotifers or brine shrimp (van der Meeren et al. 2008).

In experiment 1, massive mortality was observed from 15 to 18 DPH. Larvae were sinking, hitting against the tank bottoms, and exhibiting abnormal behavior as well as mortality. Such behaviors and mortality have been observed when ammonia reaches toxic levels. Un-ionized ammonia is considered a critical factor affecting survival, but the levels of total ammonia in this study (0.8 mg/L) were even under the safe level (1 mg/L) reported for Red Snapper *L. campechanus* (Bardon-Albaret and Saillant 2016). A similar behavior known as "walling" or "head-butting syndrome" has been reported for other species (Cobcroft et al. 2012; Madhu et al. 2016). Most likely, this behavior has been induced by the type of tanks used (black walls and white bottom) and the absence of microalgae. In black-walled tanks with a white bottom, a positive light gradient toward the bottom is established, especially in clear water, acting like a trap for marine fish larvae (Naas et al. 1996). Phototaxis in larvae has been documented in different marine fish species (Blaxter 1986). The addition of microalgae to larval tanks up to 20 DPH (experiment 2) prevented the occurrence of this behavior and greatly improved survival. The presence of microalgae creates a less stressful environment for larvae (Naas et al. 1996). However, even if drastic mortality did not occur, survival at 20 DPH in the microalgal density experiment was low and no difference was observed between Low and High microalgal densities.

Survival was enhanced by the presence of adult copepods *P. crassirostris* in larval tanks during experiment 3. However, despite a higher survival in the Adult treatment, no significant difference ($P = 0.07$) in survival was observed at 9 DPH compared to the Nauplius treatment due to the high variability among replicates. High variability in survival among replicates was also observed when small-volume tanks (20 L) were used in Yellow Tang larval culture (Pereira-Davison and Callan 2018); those authors proposed that the number of replicates should be increased. The combination of higher light intensity and the presence of copepod adults in experiment 4 also resulted in higher survival. Copepod adults provided recently hatched nauplii. Nauplius stages I and II of *P. crassirostris* last less than 6 h (Santoyo-Flores 2020), which makes the next developmental stages too large to be

ingested at first feeding by Pacific Red Snapper larvae. Anil et al. (2018) also observed higher survival when larval tanks were previously inoculated with *P. crassirostris* adults.

In conclusion, Pacific Red Snapper larvae showed a preference for *P. crassirostris* nauplii over other prey types in the first feeding days. Since these larvae have a small mouth gape, the presence of copepod adults during the fish's larval stage is important to ensure a continuous supply of stage-I–II nauplii. Moreover, adding copepod adults coupled with a light intensity of 1,000 lx increased larval survival compared to lower light intensities. Survival can also be improved by using green water during the entire duration of larval rearing since erratic larval behavior and drastic mortality were not observed when microalgae were present.

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