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Manipulating Culture Conditions and Feed Quality to Increase the Survival of Larval Marble Goby *Oxyeleotris marmorata*

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Abstract

Fish stocking density, feed quality, and tank color were evaluated for their effects on survival of larval Marble Goby *Oxyeleotris marmorata* reared at 5-g/L salinity for 30 d. Feed quality was examined in terms of proximate composition and fatty acid profile. Fish larvae were given rotifers and brine shrimp *Artemia* spp. nauplii (R-A) that were fed condensed cells of the phototrophic bacterium *Rhodovulum sulfidophilum* (bPB) cultured in palm oil mill effluent (POME); under this feeding regime, fish stocked at a density of 15 larvae/L had significantly higher survival (42.0%) than those stocked at 20 larvae/L (33.5%) or 30 larvae/L (9.9%). At the best stocking density of 15 larvae/L, fish survival and growth were further improved when the larvae received either R-A that were fed the whole culture broth of unsettled bacteria (cPB; survival = 60.9%) or R-A that were fed POME (survival = 58.6%). Under similar rearing conditions, larvae reared in gray-colored tanks showed still further significant improvement in survival (79.0%) relative to larvae that were reared in black tanks (32.5%) or transparent tanks (0.7%). Thus, optimal culture conditions for larval survival (71.4–81.9%) included rearing in gray culture tanks, stocking at 15 larvae/L, and a diet of R-A that were fed cPB or bPB. Under those optimal conditions, survival during the critical larval period (first 10 d posthatch; 91.4–95.7%) was significantly improved. An approximate 2:2:1 dietary ratio of docosahexaenoic acid : eicosapentaenoic acid : arachidonic acid as compared to a fish tissue ratio of 7:2:1 is recommended to improve the survival and production of Marble Goby larvae.

The Marble Goby *Oxyeleotris marmorata* is one of the most favored freshwater fishes in Southeast Asia due to its high commercial value and strong market demand (Luong et al. 2005). Although East Asia (i.e., China, Japan, Hong Kong, and Taiwan) is the primary market for Marble Goby, these fish have now found their way into the United States and European Union markets as live or chilled food fish and as pets (Viet Asia Foods Company 2014). Unfortunately, the large-scale culture of

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Marble Goby depends heavily on wild-caught larvae that are becoming short in supply, whereas the numbers of hatchery-produced larvae are insufficient due to failure in establishing a stable culture technique. As a result, in Southeast Asia, where the fish is mainly cultured, the current total production of Marble Goby is only 684 metric tons (FAO 2010).

Considerable research on the hatchery production of larval Marble Goby was conducted by Tavarutmaneegul and Lin (1988), Amornsakun et al. (2003), and Senoo et al. (2008); however, none of those studies examined the use of palm oil mill effluent (POME) or its products as fish feed. Compared with survival and growth reported by Senoo et al. (1994a, 2008), Loo et al. (2013a) were able to significantly improve survival and growth of larval Marble Goby by administering a diet primarily based on *Rhodovulum sulfidophilum*, a phototrophic bacterium (PB) that is grown in POME. Loo et al. (2013a) successfully raised Marble Goby larvae to 30 d posthatch (dph) in 5-g/L salinity by providing a diet of rotifers followed by brine shrimp *Artemia* spp. nauplii (after day 20); both the rotifers and *Artemia* were cultured by feeding condensed PB cells. Loo et al. (2013a) obtained significantly higher fish survival (42.5–51.6%) as compared with the best survival (16%) reported by Senoo et al. (2008) for larvae that received microalgae-fed rotifers. Despite their achievement toward establishing a stable, cost-effective culture technique for larval Marble Goby, Loo et al. (2013a) concluded that larval survival for commercial production might be further improved by manipulating feed quality and culture conditions, such as fish stocking density and tank color.

Marble Goby larvae have been cultured by using a stocking density of 14 larvae/L (Senoo et al. 2008) or 10 larvae/L (Loo et al. 2013a). Although a higher stocking density will potentially increase the number of cultured fish, it has always resulted in higher larval mortality and poor growth due to larval stress (Tort et al. 1996) and the deterioration of water quality (Biswas et al. 2006). Thus, the optimal fish stocking density varies depending on the cultured species: for instance, a density of 30 larvae/L is favorable for Turbot *Scophthalmus maximus* but not for Atlantic Halibut *Hippoglossus hippoglossus*, which must be stocked at densities less than 10 larvae/L (Shields 2001).

The relative benefits of PB and POME to rotifer nutrition have been studied (Loo 2012; Loo et al. 2013b), and the findings indicate that rotifers of different nutritional quality can result from the additive effects of PB and POME. Hence, the feeding regime used for rotifers could influence the quality of rotifers, which in turn could affect larval fish survival and growth.

To date, there has been no report on the best tank color for rearing Marble Goby larvae. Like most fish larvae, larval Marble Goby are visual feeders, and their ability to survive when switching from endogenous feeding to exogenous feeding is dependent on the availability of easily detectable prey (Ina and Higashi 1979; Blaxter 1986). Conditions that optimize the contrast between the environment and prey would enhance prey detection and capture by larvae, leading to good survival and growth (Downing and Litvak 1999). Many studies have reported that fish and crustacean larvae survive well when cultured in black or dark-colored tanks (e.g., Martin and Peterson 1998; Abed and Zeng 2005; Jennifer and Stephen 2009). Senoo et al. (2008) failed to obtain good survival and growth of Marble Goby larvae cultured in transparent tanks, whereas Loo et al. (2013a) observed good survival and growth of larvae in black tanks. Higher survival of Marble Goby in darker tanks may be related to the fact that wild Marble Goby live in a dark environment at the bottoms of rivers, ponds, and lakes; they lay their eggs at the bottom and are nocturnally active. Therefore, it is hypothesized that Marble Goby larvae will survive better in dark-colored tanks than in light-colored tanks. We tested this hypothesis in the present study.

Hence, the objectives of this study were to investigate whether the production of Marble Goby larvae can be enhanced by manipulation of larval stocking density, the nutritional quality of larval food (rotifers plus *Artemia* nauplii [R-A]), and tank color.

METHODS

Culture of phototrophic bacteria.—A 100-mL quantity of stock *Rhodovulum sulfidophilum* cultured and maintained in synthetic 112 medium (Gest and Favinger 1983) was inoculated into 900 mL of 25% diluted POME in distilled water (volume/volume). The PB-inoculated culture was then incubated under light-anaerobic conditions at 30 $\pm$ 2°C for 2 d before harvesting the bacterial culture broth.

Culture of fish.— Fish were cultured at the Marine Culture Unit (hatchery) of the University of Malaya. Marble Goby adults of the F_1 generation were used as broodstock. After spawning, the female fish was immediately removed from the tank, leaving the male to tend the eggs. The spawned eggs hatched after 2 d. Larvae were then removed and reared in either 30-L or 150-L cylindrical tanks with conical bottoms; corresponding working volumes were 25 and 100 L of water, respectively, at 5-g/L salinity. This salinity was previously found to be the best salinity for Marble Goby larviculture (Loo et al. 2013a). The required salinity was prepared by diluting seawater with aged tap water before ultraviolet irradiation. The larvae were reared for a 30-d period, during which a 30% water exchange was performed every 2 d by siphoning out the culture water through 40-μm-mesh netting and then refilling the tank with sterilized, clean water (5-g/L salinity) through 10-μm-mesh netting. Water quality characteristics, such as pH, temperature (°C), dissolved oxygen (DO) concentration (mg/L), salinity (g/L), and conductivity (mS/cm), were measured daily using the appropriate YSI meters (Model 100 pH/temperature meter, Model 550A DO meter, and Model EC300 conductivity/salinity meter; YSI, Yellow Springs, Ohio). Every 10 d, sufficient culture water was sampled from each larval tank for analyses of ammonia nitrogen (mg/L), nitrate-nitrogen (mg/L), and nitrite-nitrogen (mg/L) concentrations, which were determined using the salicylate, cadmium

reduction, and diazotization methods, respectively, for the Hach DR/2010 spectrophotometer (Hach, Loveland, Colorado).

Fish feeding.— The stock culture of the rotifer *Brachionus rotundiformis* (S type) was obtained from the Department of Fisheries Malaysia; the *Artemia* nauplii were hatched from commercially produced cysts (Hong Da *Artemia* Cysts, China). These organisms were cultured as fish food at 5-g/L salinity in 150-L, cylindrical black tanks with conical bottoms. The cultured POME-grown PB were prepared into two forms for R-A feeding: (1) the condensed bacterial cell form (bPB), which was obtained by centrifuging the culture broth of POME-grown PB at 2,300 $\times$ *g* for 20 min at 4°C, followed by rinsing the bacterial cells with a 0.9% solution of sterilized sodium chloride; and (2) the whole culture broth of unsettled POME-grown PB (cPB), which contained both bacteria and residual POME. The bPB and cPB feed preparations and POME were provided to the rotifers and *Artemia* nauplii to represent three feed types with different forms and differing nutritional quality (Loo 2012; Loo et al. 2013b). The bPB, cPB, and POME were fed to R-A at the daily rate of 1 L (~11-g dry weight). The cultured R-A were then fed to larval Marble Goby.

Prior to use in feeding the fish larvae, cultured rotifers were initially sieved through 200-µm-mesh netting followed by 40-µm-mesh netting to separate the rotifers from their culture water and from unwanted debris. Two-day-old *Artemia* nauplii (similarly fed either the bacteria or POME) were harvested using 150-µm-mesh netting. Cultured R-A were then rinsed twice with sterilized water (5-g/L salinity) prior to larval feeding. The density of rotifers administered to Marble Goby larvae was 10 individuals/mL of culture water from day 0 to day 20 and 5 individuals/mL from day 21 to day 30; *Artemia* were offered at a density of 5 nauplii/mL from day 21 onwards. Marble Goby larvae at an age of 1 dph were used in all experiments described below.

Experiment 1.— A previous study (Loo et al. 2013a) showed that Marble Goby larvae survived well at a stocking density of 10 larvae/L, whereas all died after being stocked at a density of 33 larvae/L; therefore, experiment 1 was designed to evaluate the effects of three intermediate stocking densities (15, 20, and 30 larvae/L) and day of culture (days 10, 20, and 30) on larval survival and growth. The larvae were reared in 150-L black-colored tanks, with each treatment conducted in triplicate. Twice daily, the larvae were fed R-A cultured from bPB.

Experiment 2.— The second experiment evaluated the effects of feed form and quality (R-A that were fed bPB, cPB, or POME) and day of culture (days 10, 20, and 30) on the survival and growth of Marble Goby larvae. The larvae (1,500 fish; 15 larvae/L) were stocked into 150-L, black-colored tanks, with triplicate tanks for each treatment.

Experiment 3.— The third experiment determined the effects of tank color (transparent, gray, and black) and day of culture (days 10, 20, and 30) on larval survival and growth. Since tank color likely influences the interior light conditions due to natural light from the top and through the tank walls, a HOBO pendant temperature/light data logger (Model UA-002-64; Onset Computer, Bourne, Massachusetts) was placed inside the interior of each tank (with water) to measure the relative light intensities. Larvae (375 fish; 15 larvae/L) were reared in 30-L tanks, with triplicate tanks for each treatment. The larvae received cPB-cultured R-A as food.

Experiment 4.— The final experiment tested the effects of feed form and quality (R-A fed with cPB or bPB) and day of culture (days 10, 20, and 30) on the survival and growth of larval Marble Goby cultured in gray tanks. The larvae (375 fish; 15 larvae/L) were reared in 30-L tanks, with each treatment conducted in triplicate.

Survival and growth of Marble Goby larva.— The number and TL (mm) of surviving Marble Goby larvae were determined at 10-d intervals. Before stocking, nine larvae from the same brood were sacrificed and measured for initial TL. On each sampling day, culture water in the larval tanks was completely drained to facilitate counts of the surviving larvae. The larvae were then returned to their culture tanks after the tanks were refilled with fresh, 5-g/L-salinity water. Three larvae per tank were sacrificed, and their TLs were measured under a dissecting microscope (Leica MZ8) with the aid of an image analysis system (Moticam 2500). The small sample size taken was due to concerns over high mortality at the early rearing stage (Tan and Lam 1973; Tavarutmaneegul and Lin 1988) and handling-induced mortality. Survival at 10, 20, and 30 d of culture was calculated based on the start of larval culture (day 0).

Biochemical analyses.— For biochemical analyses, samples of bPB- and cPB-fed rotifers were harvested after 96 h of culture, and samples of Marble Goby larvae that fed on rotifers from these groups were harvested after 15 d of culture. The samples were thoroughly rinsed with filtered distilled water and were immediately freeze-dried prior to analyses.

Standard methods from the Association of Official Analytical Chemists (AOAC 1995) were used to determine the protein (AOAC 981.10), fat (AOAC 991.36), ash (AOAC 923.03), and moisture (AOAC 950.46) content of freeze-dried rotifers and Marble Goby larvae. Carbohydrate was determined according to the method of Pomeranz and Meloan (1987). Fatty acid methyl esters were prepared in accordance with method 2.301 of the International Union of Pure and Applied Chemistry (IUPAC 1987). A gas chromatograph (Hewlett-Packard HP5890) was used to analyze the fatty acid profile of freeze-dried rotifers and Marble Goby larvae. The Waters AccQ-Tag method (Waters, Milford, Massachusetts) based on high-performance liquid chromatography was used to determine the amino acid profile of the samples. Fatty acid, amino acid, and proximate analyses were performed on triplicate samples per treatment.

Statistical analysis.— Survival and TLs of Marble Goby larvae in each treatment were averaged, and SDs were then calculated. Prior to parametric analysis, the percent survival results in all experiments were arcsine transformed.

The percent survival and TL of Marble Goby larvae on the final day of the experiment (day 30) as affected by stocking density, feed quality, or tank color were tested by using one-way ANOVA (experiments 1–3) for multiple groups or by using Student's *t*-test (experiment 4) for two groups. Each variable resulting from biochemical analyses of bPB- and cPB-fed rotifers and the Marble Goby larvae that were given those rotifer groups as feed was tested for a significant difference by using a *t*-test. The environmental (pH, DO, conductivity, and temperature) and water quality (ammonia nitrogen, nitrite-nitrogen, and nitrate-nitrogen) variables measured at days 10, 20, and 30 were averaged for each treatment (fish stocking density, feed form and quality, and tank color), and the SDs were calculated. Statistical analysis was performed by using Statistica version 10, with $P \leq 0.05$ taken as significant to reject the null hypothesis for each test. The fatty acid and amino acid profiles of rotifers and Marble Goby larvae were interpreted using principal components analysis, which was run on CANOCO version 4.5 (Ter Braak and Smilauer 2002).

RESULTS

Experiment 1: Effect of Stocking Density on Survival and Growth of Marble Goby Larvae Cultured in Black Tanks

After 30 d of culture, the survival of Marble Goby larvae that received R-A cultured from bPB was lowest (9.9%) at the highest stocking rate of 30 larvae/L (one-way ANOVA: $P < 0.01$) relative to survival at a density of 15 larvae/L (41.9%) or 20 larvae/L (33.5%; Table 1). The latter two survival rates were not significantly different ($P > 0.05$). For all stocking densities, most of the larval mortality (36.5–57.1%) occurred during the first 10 d of culture, whereas mortality decreased as the larvae grew. Larval mortality was lowest during the final 10 d of culture (days 20–30), especially for the lowest stocking density (1.2%) in comparison with the highest density (5.8%).

The mean TL of 30-dph Marble Goby larvae at the stocking density of 15 larvae/L (10.97 mm) was significantly greater ($P < 0.01$) than the mean TL observed at a density of 20 larvae/L (9.74 mm) or 30 larvae/L (9.02 mm). Average growth rates over 30 d of culture were 0.24, 0.20, and 0.18 mm/d for the 15-, 20-, and 30-larvae/L density treatments, respectively. Hence, based on the higher survival and growth observed at a stocking density of 15 larvae/L, this density was used in all subsequent experiments.

Experiment 2: Effect of Feed Form and Quality on Survival and Growth of Larvae Cultured in Black Tanks

After 30 d of culture, mean survival of Marble Goby larvae was not significantly different among groups that were fed R-A cultured using cPB (survival = 60.9%), POME (58.6%), or bPB (50.9%; one-way ANOVA: $P > 0.05$; Table 2). During the first 10 d of culture, larvae that received cPB-cultured R-A had lower mortality (1.8%) than larvae that were fed POME-cultured R-A (16.4%) or bPB-cultured R-A (34.6%). However, during the final 10 d of culture, larvae that were given bPB-cultured R-A had lower mortality (8.2%) than larvae that were fed POME-cultured R-A (8.8%) or cPB-cultured R-A (17.5%).

The mean TL of 30-dph Marble Goby larvae that received bPB-cultured R-A (11.07 mm) was also not significantly different (one-way ANOVA: $P > 0.05$) from the mean TLs of larvae that were fed POME-cultured R-A (9.91 mm) or cPB-cultured R-A (9.74 mm). The average growth rates were 0.24, 0.20, and 0.19 mm/d for larvae that received R-A cultured using bPB, POME, and cPB, respectively.

Experiment 3: Effect of Tank Color and Light Intensity on Larval Survival and Growth

The different tank colors provided light intensities of 2,942 $\pm$ 2,309 lx for transparent tanks, 1,772 $\pm$ 630 lx for gray tanks, and 872 $\pm$ 819 lx for black tanks at 1145 hours, when the ambient light in the hatchery was 11,137 $\pm$ 3,009 lx.

At the end of the culture period, the mean survival of Marble Goby larvae that were fed cPB-cultured R-A was significantly different among the three tank color/light intensity treatments (one-way ANOVA: $P < 0.01$). Survival was highest in gray tanks (79.0%), intermediate in black tanks (32.5%), and lowest in transparent tanks (0.7%; Table 3). During the first 10 d of culture, larvae reared in gray tanks had the lowest mortality (6.2%) relative to larvae that were reared in transparent tanks (52.5%) or black tanks (41.2%). As the larvae grew, mortality generally decreased.

Mean TL at 30 dph was not significantly different (*t*-test: $P > 0.05$) between larvae reared in black tanks (10.91 mm) and those reared in gray tanks (11.44 mm; Table 3). Total lengths of 20-dph and 30-dph larvae cultured in transparent tanks were not measured because there were very few surviving larvae. The average growth rates of larvae reared in black tanks and gray tanks were 0.24 and 0.25 mm/d, respectively.

Experiment 4: Effect of Feed Form and Quality on Survival and Growth of Larvae Cultured in Gray Tanks

The prior experiments revealed that Marble Goby larvae survived best when cultured in gray tanks and stocked at a density of 15 larvae/L. Thus, given these optimal conditions, the final experiment evaluated which feed form and quality (i.e., R-A cultured from cPB or bPB) could provide further improvements in larval survival.

For the entire 30-d culture period, the overall mean survival of larvae that were fed cPB-cultured R-A (81.9%) was not significantly different (*t*-test: $P > 0.05$) from the survival of larvae that received bPB-cultured R-A (71.4%; Table 4). Both feed types were associated with a low mortality rate (4.3–8.6%) of larvae during the first 10 days of culture. Mortality increased slightly by day 20 (11.2–14.7%) but fell thereafter (day-30 mortality: 2.6–5.3%).

Mean TL at 30 dph was significantly greater (*t*-test: $P < 0.05$) for larvae that were given bPB-cultured R-A (12.30 mm)

TABLE 1. Effects of three stocking densities (experiment 1) on the mean (± SD) number of surviving larvae, survival (%), and TL (mm) of Marble Goby cultured in black tanks for 30 d. Larvae were given a diet of rotifers (days 0–30) and *Artemia* nauplii (days 21–30), both of which were fed the condensed biomass of *Rhodovulum sulfidophilum* cultured in palm oil mill effluent (i.e., bPB). Survival and TL at day 30 were tested for significant differences among treatments by using one-way ANOVA; means with the same letters are not significantly different ($P > 0.05$).

Fish stocking density (larvae/L)	Day of culture	Number of surviving larvae[a]	Larval survival (%)[a]	TL (mm)[b]
15	0	1,500 ± 0	100.0 ± 0.0	3.77 ± 0.21
	10	806 ± 258	53.7 ± 17.2	5.88 ± 0.22
	20	646 ± 176	43.1 ± 11.7	9.04 ± 0.49
	30	629 ± 184	41.9 ± 12.3 z	10.97 ± 0.43 w
20	0	2,000 ± 0	100.0 ± 0.0	3.77 ± 0.21
	10	1,270 ± 178	63.5 ± 8.9	5.61 ± 0.55
	20	701 ± 165	35.1 ± 8.3	8.22 ± 0.59
	30	669 ± 171	33.5 ± 8.5 z	9.74 ± 0.19 v
30	0	3,000 ± 0	100.0 ± 0.0	3.77 ± 0.21
	10	1,288 ± 402	42.9 ± 13.4	5.39 ± 0.32
	20	471 ± 290	15.7 ± 9.7	6.55 ± 0.46
	30	297 ± 155	9.9 ± 5.2 y	9.02 ± 0.49 v

[a]Means of triplicate tanks per treatment. Survival was calculated based on initial stocking density.
[b]Means of nine values (3 specimens/tank) from each treatment.

than for those that were fed cPB-cultured R-A (11.07 mm). Average growth rates for the two treatment groups were 0.29 and 0.24 mm/d, respectively.

Water Quality Characteristics

The water quality variables measured in larval tanks were not significantly different among treatments or among experiments ($P > 0.05$). In all four experiments, recorded ranges were 6.98–8.38 for pH, 4.22–6.11 mg/L for DO, 9.09–9.96 mS/cm for conductivity, 26.8–30.0°C for temperature, 0.02–0.47 mg/L for ammonia nitrogen, 0.010–0.327 mg/L for nitrite-nitrogen, and 0.19–0.84 mg/L for nitrate-nitrogen (Table 5).

Biochemical Composition of Freeze-Dried Rotifers and Marble Goby Larvae

Rotifers that were fed cPB had higher percentages of protein, fat, and energy than rotifers that were cultured using bPB; the

TABLE 2. Effects of feed form and quality (experiment 2) on the mean (± SD) number of surviving larvae, survival (%), and TL (mm) of Marble Goby cultured in black tanks at a stocking density of 15 larvae/L for 30 d. Feed forms were rotifers and *Artemia* nauplii (R-A) cultured by feeding with (1) the condensed biomass of bacteria *Rhodovulum sulfidophilum* (bPB) cultured in palm oil mill effluent (POME); (2) the whole culture broth of bacterial cells (cPB) in POME; and (3) POME only. Survival and TL at day 30 were tested for significant differences among treatments by using one-way ANOVA; means with the same letters are not significantly different ($P > 0.05$).

Feed form	Day of culture	Number of surviving larvae[a]	Larval survival (%)[a]	TL (mm)[b]
R-A fed with bPB	0	1,500 ± 0	100.0 ± 0.0	4.00 ± 0.10
	10	981 ± 171	65.4 ± 11.4	6.35 ± 0.28
	20	887 ± 164	59.1 ± 10.9	8.63 ± 0.15
	30	763 ± 246	50.9 ± 16.4 z	11.07 ± 0.98 w
R-A fed with cPB	0	1,500 ± 0	100.0 ± 0.0	4.00 ± 0.10
	10	1,473 ± 11	98.2 ± 0.7	5.60 ± 0.38
	20	1,177 ± 108	78.4 ± 7.2	8.68 ± 0.37
	30	914 ± 126	60.9 ± 8.4 z	9.74 ± 0.50 w
R-A fed with POME	0	1,500 ± 0	100.0 ± 0.0	4.00 ± 0.10
	10	1,255 ± 96	83.6 ± 6.4	5.19 ± 0.10
	20	1,012 ± 117	67.4 ± 7.8	8.51 ± 0.39
	30	878 ± 117	58.6 ± 7.8 z	9.91 ± 0.57 w

[a]Means of triplicate tanks per treatment. Survival was calculated based on initial stocking density.
[b]Means of nine values (3 specimens/tank) from each treatment.

TABLE 3. Effects of tank color and light intensity (experiment 3) on the mean (± SD) number of surviving larvae, survival (%), and TL (mm) of Marble Goby cultured for 30 d at a stocking density of 15 larvae/L. Larvae were given a diet of rotifers (days 0–30) and *Artemia* nauplii (days 21–30), both of which were fed the whole culture broth of *Rhodovulum sulfidophilum* cells in palm oil mill effluent (i.e., cPB). Survival and TL at day 30 were tested for significant differences among treatments by using one-way ANOVA; means with the same letters are not significantly different ($P > 0.05$).

Tank color (light intensity)	Day of culture	Number of surviving larvae[a]	Larval survival (%)[a]	TL (mm)[b]
Transparent (2,942 ± 2,309 lx)	0	375 ± 0	100.0 ± 0.0	3.83 ± 0.15
	10	178 ± 20	47.5 ± 5.5	4.52 ± 0.25
	20	5 ± 7	1.2 ± 1.9	[c]
	30	3 ± 5	0.7 ± 1.2 z	[c]
Black (872 ± 819 lx)	0	375 ± 0	100.0 ± 0.0	3.83 ± 0.15
	10	220 ± 54	58.8 ± 14.3	5.19 ± 0.04
	20	132 ± 17	35.3 ± 4.7	7.46 ± 0.97
	30	122 ± 15	32.5 ± 4.0 y	10.91 ± 0.64 w
Gray (1,772 ± 630 lx)	0	375 ± 0	100.0 ± 0.0	3.83 ± 0.15
	10	352 ± 24	93.8 ± 6.5	5.19 ± 0.44
	20	310 ± 27	82.6 ± 7.3	8.61 ± 0.15
	30	296 ± 38	79.0 ± 10.2 x	11.44 ± 0.21 w

[a] Means of triplicate tanks per treatment. Survival was calculated based on initial stocking density.
[b] Means of nine values (3 specimens/tank) from each treatment.
[c] Total lengths were not measured for the transparent tanks on days 20 and 30 due to very few surviving larvae and complete mortality in two replicate tanks at day 20.

carbohydrate percentage was higher for the bPB-cultured rotifers than for cPB-cultured rotifers, and the two groups had approximately equal amounts of ash (Table 6). The fatty acid profile revealed that cPB-cultured rotifers had significantly higher ($P < 0.05$) total polyunsaturated fatty acids (PUFAs), especially n-6 PUFAs, than bPB-cultured rotifers; however, total saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs) were lower for cPB-cultured rotifers. Furthermore, cPB-cultured rotifers had significantly higher ($P < 0.05$) amounts of docosahexaenoic acid (DHA; 22:6[n-3], where 22 is the number of carbon atoms, 6 is the number of double bonds, and 3 represents the position of the first double bond from the methyl end) and arachidonic acid (ARA; 20:4[n-6]) than bPB-cultured rotifers. The percentages of total amino acids were also significantly different between the two groups of rotifers ($P < 0.05$).

Marble Goby larvae that received rotifers fed with cPB had significantly higher percentages of carbohydrate and ash than larvae that were given bPB-cultured rotifers, but both groups of larvae had approximately equal amounts of fat and moisture (Table 6). However, larvae that were fed bPB-cultured rotifers had more protein (and total amino acids) and significantly higher ($P < 0.05$) amounts of n-3 PUFAs, especially DHA and eicosapentaenoic acid (EPA; 20:5[n-3]), than larvae that were offered cPB-cultured rotifers as feed. Larval fish of the two groups had equal total PUFA contents (Table 6).

TABLE 4. Effects of feed form and quality (experiment 4) on the mean (± SD) number of surviving larvae, survival (%), and TL (mm) of Marble Goby cultured in gray tanks at a stocking density of 15 larvae/L for 30 d. Feed forms are defined in Table 2. Survival and TL at day 30 were tested for significant differences between treatments by using one-way ANOVA; means with the same letters are not significantly different ($P > 0.05$).

Feed form	Day of culture	Number of surviving larvae[a]	Larval survival (%)[a]	TL (mm)[b]
R-A fed with cPB	0	375 ± 0	100.0 ± 0.0	3.73 ± 0.06
	10	359 ± 19	95.7 ± 5.0	5.56 ± 0.46
	20	317 ± 15	84.5 ± 4.1	9.14 ± 0.08
	30	307 ± 11	81.9 ± 3.0 z	11.07 ± 0.05 w
R-A fed with bPB	0	375 ± 0	100.0 ± 0.0	3.73 ± 0.06
	10	343 ± 42	91.4 ± 11.2	5.49 ± 0.25
	20	288 ± 89	76.7 ± 23.8	8.83 ± 0.42
	30	268 ± 77	71.4 ± 20.5 z	12.3 ± 0.52 v

[a] Means of triplicate tanks per treatment. Survival was calculated based on initial stocking density.
[b] Means of nine values (3 specimens/tank) from each treatment.