Application of the minute monogonont rotifer *Proales similis* de Beauchamp in larval rearing of seven-band grouper *Epinephelus septemfasciatus*

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Abstract

In comparison to the rotifer *Brachionus rotundiformis*, the euryhaline rotifer *Proales similis* has a much smaller body size (83 μm in length and 40 μm in width), and it may be applicable as live food for rearing marine fish larvae with a very small mouth size. A mass culture technique of *P. similis* was recently established, and it has already been confirmed that marine fish larvae could ingest *P. similis*. In the present study, we further investigated the use of *P. similis* as an initial food by observing larval ingestion and digestion and analyzing the nutritional profile, as well as through a 10-day larval rearing trial to investigate survival and growth. Seven-band grouper *Epinephelus septemfasciatus* larvae showed higher selectivity against *P. similis* than *B. rotundiformis* 4 days after hatching. The larvae digested and utilized *P. similis* as an energy resource as they grew, and survived until the end of the experiment. The fatty acid profile of *P. similis* changed according to the type of microalgae; *Nannochloropsis oculata* NIES-2146 strain and "super fresh" *Chlorella vulgaris* V-12® (Chlorella Industry, Fukuoka, Japan) were used as food sources. Higher growth and survival during the initial 10 days were observed when *P. similis* and *B. rotundiformis* were co-fed to the seven-band grouper larvae.

Keywords: Rotifer, *Proales similis, Brachionus rotundiformis,* live food, fatty acid, *Epinephelus septemfasciatus*.

1. Introduction

The euryhaline rotifer *Brachionus plicatilis* has been used as an excellent initial live food for rearing marine fish larvae (Ito, 1960; Lubzens, 1987; Lubzens et al., 1989; Hagiwara et al., 2001, 2007). *B. plicatilis* is a complex of sibling species with a body length ranging between 90 and 400 μm. Among these species, *B. rotundiformis* (Segers, 1995; Ciros-Perez et al., 2001; Gomez et al., 2002; Kotani et al., 2005; Fontaneto et al., 2007) is the smallest in size and is commonly referred to as a "super-small" (SS) -type by culturists. *B. rotundiformis* has been used for feeding fish larvae with small mouths, such as groupers (Okumura, 1997; Soyano et al., 2008). It is reported that grouper larvae at the initial stage (*Epinephelus akaara*, *E. septemfasciatus*, *E. bruneus*, *Plectropomus leopardus*) ingest smaller (or younger) *B. rotundiformis*, which contain less nutrition because they hatch in larval rearing tanks and feed on background microalgae added to the tanks at a low density (Okumura, 1997). Furthermore, there are tropical marine fish species such as Napoleon wrasse (*Cheilinus undulatus*) and angelfishes (family Pomacanthidae), whose larvae cannot ingest *B. rotundiformis* because of their extremely small mouth size (Tucker, 1998), and they require even smaller live food in the range 40-80 μm at the initial feeding stages (Slamet and Hutapea, 2004; Olivotto, et al., 2006). Many wrasses (family Labridae) including *C. undulatus* are economically important because of their high market prices, especially in Asian countries. Marine angelfish are commercially valuable as ornamental fish.

However, mass larval rearing techniques have not been established for these groups, partly due to the lack of appropriate live feed for their initial feeding.

Recognizing these demands for smaller-sized live feed, we have developed *Proales similis* as a starter live food organism for the larviculture of small mouth fishes (Wullur et al., 2009). The average body size of *P. similis* (83 μm in length and 40 μm in width) is 38% smaller and 60% narrower than the so-called SS-type Indonesian strain *B. rotundiformis* (Hagiwara et al., 1995; Wullur et al., 2009). *P. similis* can be mass-cultured in the same manner as euryhaline *Brachionus* (Wullur et al., 2009). We also confirmed that marine fish larvae, namely the seven-band grouper *Epinephelus septemfasciatus* larvae, could ingest *P. similis* (Wullur et al., 2009).

In the present study, we further investigated the use of *P. similis* as an initial food by monitoring the ingestion, digestion, survival and growth of seven-band grouper to determine whether the feeding of *P. similis* is effective to enhance the survival and growth of the larvae, although larval rearing in this species is conducted by feeding so-called S or SS-type rotifers (Hagiwara et al., 2007; Soyano et al., 2008). We also conducted an analysis of the fatty acid profile of *P. similis* to examine whether *P. similis* can be nutritionally manipulated as well as whether larvae can utilize *P. similis* as a nutritional source so that larvae show appropriate survival and growth.

2. Materials and Methods

2.1. Rotifer culture

The *P. similis* used in this study was originally collected from an estuary in Ishigaki Island, Okinawa, Japan, in July 2004 (Wullur et al., 2009). *P. similis* was mass-cultured in 50 l polycarbonate tanks. The water temperature and salinity were 25 ^{o}C and 25 ppt, respectively. Concentrated microalgae *N. oculata* NIES-2146 strain and "super fresh" *Chlorella vulgaris* V-12® (reviewed by Hagiwara et al., 2001), purchased from Chlorella Industry Fukuoka, Japan, were fed to the rotifers. Mass culture and nutritional enrichment were performed at the same time with these microalgae, and harvested rotifers were directly fed to fish larvae. The two microalgal species were supplied to rotifers twice a day to maintain the density at 28.8 μg dry weight/ml, which corresponds to $12.5x10^6$ and $4.3x10^6$ cells/ml for *N. oculata* and *C. vulgaris* V-12®, respectively. These food levels are optimal for the mass culture of *P. similis* (Wullur et al., 2009). Rotifer population density was monitored once daily by counting the number of rotifers in triplicate 1-ml samples taken from each culture tank. During the larval rearing experiment, *P. similis* was raised (in 30-50 l) using the semi-continuous culture method (reviewed by Hoff and Snell, 1987; Lubzens, 1987) by replacing 1/3 of the culture water. The size distribution of each rotifer species was measured using a digital microscope (Keyence VH-8000, Keyence Corp.) at a magnification of 450x, while dry weight was determined as follows: each rotifer was debris-free siphoned from each

culture and filtered using a 10-μm mesh plankton net to remove microalgae. The filtered rotifers were transferred to a beaker containing 250 ml diluted seawater (25 ppt) in three replications, and aliquot samples were counted to estimate the total number of each rotifer species. Further, the rotifers were re-filtered using previously weighed precombusted Whatman GF/C fiberglass filters and rinsed with distilled water to remove salt. The filters were dried overnight at 80 ^{o}C, and dry weight was determined gravimetrically using a microbalance (Mettler Toledo UMX2, Mettler-Toledo, Columbus, Ohio, USA).

2.2. Larval rearing of seven-band grouper larvae

Feeding trials of seven-band grouper *E. septemfasciatus* larvae were conducted in 9 transparent 100-l polycarbonate cylindrical tanks following the method described by Ruttanapornvareesakul et al. (2007). The tanks were placed in the laboratory, where the temperature (24-26 ^{o}C) and photoperiod (12L:12D) were constant. Artificial seawater at a salinity of 32-34 ppt (Marine Art Hi, Tomita Pharmaceutical, Japan) was used to fill the tanks. The rearing water was not exchanged throughout the experiment. Ceramic sand (MS-0, Norra Co. Ltd., Kyoto, Japan) was placed on the bottom of each tank to help stabilize the water quality. Aeration at a rate of 50 ml/min controlled by a flow meter (Kofloc RK-1350V) was provided through an air stone placed at the center of the bottom of each tank.

The rearing experiment was conducted using artificially fertilized eggs of *E. septemfasciatus* obtained from Nagasaki Prefectural Fisheries Experimental Station on May 24, 2007. Each tank was stocked with 1,500 eggs. Oil was applied at 0.2 ml/m^2 to form a film on the surface of each rearing tank to prevent surface death of the larvae (Yamaoka et al., 2000). At the onset of feeding at 4 days after hatching (DAH), rotifers were introduced in three treatments, each with three replicates. The three feeding treatments were 20 ind./ml *P. similis*, 20 ind./ml *B. rotundiformis* and a mixture of both rotifer species (each at 10 ind./ml), respectively. Rotifers were first enriched with "super fresh" *C. vulgaris* V-12® for around 1 week prior to the feeding of the larvae. After the addition of the rotifers, "super fresh" *C. vulgaris* V-12® at $5x10^5$cell/ml was introduced to the larval rearing tanks for green water culture. The densities of the rotifers as well as "super fresh" *C. vulgaris* V-12® inside the larval rearing tanks were replenished daily to maintain experimental levels.

Seven-band grouper larvae were reared for 10 days after hatching. At 4, 5, 6, 8 and 10 DAH, 10 larvae were sampled from each tank between the hours of 1600 and 1700, anesthetized with 0.01% MS 222 (Tricaine; Sigma Chemical Co., St. Louis, MO, USA) and fixed with 5 % formaldehyde. The mouth size (upper jaw length times $2^{0.5}$, Shirota, 1970) and standard (notochord) length of the larvae were measured using a digital microscope (VH-6300, Keyence Corp., Japan) at a magnification of 100-175 x. Feeding incidence (percentage of larvae with rotifers in the gut) and food quantity

(number of rotifers in the gut) were measured by dissecting the larval gut under a stereomicroscope. Food quantity was determined by counting the number of undigested rotifer bodies as well as the trophi of digested rotifers (Akazawa et al., 2008). Trophi are the calcified jaws located at the beginning of the digestive tracts of rotifers (Kleinow et al., 1990; Kleinow, 1998; Sorensen, 2002), and they remain undigested after the other relatively soft parts of the rotifer have been digested, thus providing a more accurate estimation of food quantity. The survival rate of the larvae in all treatments was estimated by counting the number of surviving larvae at the end of the experiment.

2.3. Biochemical analysis

2.3.1 Tryptic enzyme analysis for seven-band grouper larvae

The tryptic enzyme activity of seven-band grouper larvae was measured using a methods that followed that of Ruttanapornvareesakul et al. (2010) with slight modifications of Ueberschar (1995) and Araujo et al., (2001). Five larvae were sampled early in the morning prior to feeding at 5, 8 and 10 DAH, anesthetized with 0.01% MS 222 (Tricaine; Sigma Chemical Co., St Louis, MO, USA) and then frozen at –80 ^{o}C until analysis. For the measurement of enzyme activity, the larvae were individually homogenized with 1 ml artificial seawater (34 ppt) using a sonicator (Sonifier 150®, Branson Ultrasonics Corporation, Danbury, USA) in an ice-cold bath. The aliquot samples of the same larva were treated with 20 µl of enzyme substrate (Boc-Phe-Ser-

Arg-MCA) using a repeating pipet and syringe tip (Nichimate Stepper), and then were vortexed well using a tube mixer (Ms1 Minishacker, Kika Works (Asia) Sdn. Bhd., USA) before being placed in an incubator at 37 ^{o}C in darkness for 15 minutes. Further, 20 μl of 0.5M SDS (sodium dodecyl sulfate) was added to the samples, and they were then centrifuged at 9,000 rpm for 5 minutes at 4 ^{o}C (Kubota 6900, Tokyo, Japan). One hundred μl of the supernatant of the centrifuged samples and standard fluorescent product were placed into 96 multi-well microplates to measure the tryptic activity, which was determined by the change in the fluorescent product at excitation (360 nm) and emission (460 nm) using a fluorescence multi-well plate reader (Cytofluor Series 4000®, Applied Biosystems, CA, USA). Sterilized artificial seawater (34 ppt) was used as a blank sample for the measurement.

2.3.2 Fatty acid analysis

For the analysis of fatty acid composition, *P. similis* and *B. rotundiformis* samples were collected during the exponential growth phase and concentrated using a nylon plankton net with a 10-μm mesh size. The concentrated rotifer was washed in running tap water to remove salt, rinsed with distilled water, dried from beneath the net using filter paper and stored at –80 ^{o}C prior to analysis. The total lipids and fatty acid composition of the samples were analyzed at a commercial laboratory (Chlorella Industry Co., Fukuoka, Japan) following the method of Folch et al. (1957). The rotifer

methanolysates were prepared under 100 ^{o}C for an hour after the addition of 2M hydrogen chloride methanol. Fatty acid methyl esters (FAME) were extracted by petroleum ether. Gas chromatography analysis was performed using a GC-14A (Shimadzu Scientific Instruments, Inc.) equipped with a HR-SS-10 column. The column temperature was regulated at 150 to 220 ^{o}C. Individual fatty acids were quantified by means of the response factor to pentadecanoic acid methyl ester (GL Sciences Inc.) as the internal standard, which was added after the FAME extraction. As a reference, a similar procedure of mass culture and fatty acid analysis was performed with the *B. rotundiformis* Indonesian strain (Hagiwara et al., 1995; Wullur et al., 2009).

2.4. Statistical analysis

The differences between means in food quantity, tryptic activity, growth and survival of the larvae were first analyzed using one-way ANOVA (p<0.05) and further analyzed using the Tukey-Kramer test (p<0.05) if a difference was detected. All data in percentage or ratio terms were arcsine-square-root transformed prior to the analysis. The feeding selectivity of the larvae on two rotifer species in mixed feeding treatments was analyzed using Chesson's selectivity index (α_i):

$$\alpha_i = (r_i/p_i) / \Sigma(r_i/p_i)$$

where r_i is the frequency of prey *i* in the larval gut, and p_i is the frequency of prey *i* in the environment. This index, α_i, varies between 0 and 1 with $\alpha_i = 0.5$ indicating non-selective feeding towards prey *i*, $\alpha_i > 0.5$ indicating a preference for prey *i*, and $\alpha_i < 0.5$ indicating discrimination against prey *i*. Significant differences in selectivity between two rotifer species were analyzed using Student's *t*-test to compare the selection of a specific rotifer species to the natural selection by using the equation in Chesson (1983):

$$t = \frac{\alpha_i - 0.5}{\sqrt{s^2 / K}}$$

where α_i is the sample mean and s^2 is the sample variance of the *K* estimates of α_i.

3. Results

3.1. Ingestion and food selectivity

The body size and dry weight of the *P. similis* and *B. rotundiformis* used in the present study are presented in Table 2. Mouth opening occurred in the seven-band grouper larvae during the night at 3 DAH, and the mouth size of the larvae at 4 DAH was 180±20 μm (n=10). The larvae at 4 DAH showed active feeding on both rotifer species just after the addition of rotifers into the rearing tanks, and all larvae at 4 DAH ingested rotifers in all dietary treatments (100% feeding incidence). The numbers of rotifers in the guts of the larvae were significantly different among treatments except at 10 DAH (ANOVA, $p<0.05$). The feeding quantity of the larvae in the combined treatment was not significantly different from that of the larvae fed *P. similis* alone, but it was significantly higher than that of the larvae fed *B. rotundiformis* alone from 4 to 8 DAH (Tukey-Kramer test, $p<0.05$, Fig. 1). The larvae co-fed 2 rotifer species significantly selected *P. similis* over *B. rotundiformis* at 4 DAH, but the selection shifted to *B. rotundiformis* from 6 DAH (Student *t*-test, $p<0.05$, Fig. 2).

3.2. Digestion

The tryptic enzyme activity of seven-band grouper larvae in all dietary treatments was not significantly different except at 10 DAH (ANOVA, $p<0.05$). On this day (10 DAH), the tryptic enzyme activity of the larvae receiving the co-feeding

treatment was significantly higher than in the single-species feeding treatments (Tukey-Kramer, $p<0.05$). The tryptic enzyme activities among the larvae in the latter two treatments were not significantly different (Tukey-Kramer, $p<0.05$) (Fig.3).

3.3 Fatty acid composition

Total lipids per wet weight of *P. similis* fed with the *N. oculata* NIES-2146 strain and "super fresh" *C. vulgaris* V-12® were 2.4 and 2.6%, respectively. The fatty acid composition in the total lipids of *P. similis* changed according to the microalgae. The relative levels of essential fatty acids (EFA) for marine fish larvae such as eicosapentaenoic acid (EPA, 20:5*n*-3), docosahexaenoic acid (DHA, 22:6*n*-3) and arachidonic acid (ARA, 20:4*n*-6) in the total lipids of *P. similis* cultured by the *N. oculata* NIES-2146 strain were 23.2, 0.0 and 5.3%, respectively, while these were 11.0, 17.5 and 0.5%, respectively, when "super fresh" *C. vulgaris* V-12® was fed to the rotifers (Table 1). The levels in *B. rotundiformis* fed "super fresh" *C. vulgaris* V-12® were 5.8, 6.1 and 1.2%, respectively (Table 1). The ratios of DHA/EPA in two rotifer species fed "super fresh" *C. vulgaris* V-12® were 1.59 and 1.05 for *P. similis* and *B. rotundiformis*, respectively.

3.4. Larval rearing

The growth of the larvae was significantly different among treatments during the experiment (Fig. 4; ANOVA, $p<0.05$). The growth of the larvae at 4 to 6 DAH was significantly greater in the combined treatment than in the single-species feeding treatments (Tukey-Kramer, $p<0.05$). From 8 to 10 DAH, growth was higher in the treatment with *B. rotundiformis* alone and in the combined treatment than in the treatment with *P. similis* alone (Tukey-Kramer, $p<0.05$).

The larvae co-fed *P. similis* and *B. rotundiformis* showed higher survival (14.3 %) than those fed *P. similis* alone (2.7 %) at the end of experiment at 10 DAH (Fig. 5). Larvae fed *B. rotundiformis* alone showed intermediate survival (6.4 %, Fig. 5).

4. Discussion

The body size of *P. similis* in the present study was consistent with the previous report (Wullur et al., 2009). The dry weight of *P. similis* (35.9±7.8 ng individual^{-1}) was 6-fold lower than that of *B. rotundiformis*. The effects of two rotifers on the seven-band grouper larvae in terms of survival, growth and feeding activity were tested based on rotifer density instead of energy content because rotifer density is more informative from a practical perspective for hatchery operators, who usually feed larvae based on rotifer density.

Seven-band grouper larvae actively ingested *P. similis* just after mouth opening at 4 DAH. Thereafter, 100% feeding incidence was observed in all treatments until the end of the experiment. The number of rotifers in the larval gut was higher in the larvae receiving the treatment containing *P. similis* than in those fed *B. rotundiformis* alone (Fig. 1). This may be because *P. similis* is much smaller than *B. rotundiformis* and the larvae could consume more *P. similis* to fill the available space in their guts. When 2 rotifer species were co-fed to the grouper larvae, Chesson's selectivity index indicated that the grouper larvae showed higher selectivity for *P. similis* at 4 DAH (Fig. 2). This preference became neutral at 5 DAH, and the larvae switched their preference to larger rotifers (*B. rotundiformis*) after 6 DAH. It has been suggested that size, motion and color (Utne-Palm, 1999; Shaw et al., 2003; Tanaka et al., 2006; Akazawa et al., 2008) are among the characteristics according to which fish larvae select live food. Our observations indicated an apparent similarity in the swimming behavior and color of the two rotifer species (unpublished); thus, it is likely that the cause of the higher prey selection of *P. similis* by grouper larvae during early feeding (Fig. 2) is attributable to this rotifer's smaller body size. The change of preference by the larvae to a larger rotifer (*B. rotundiformis*) after 6 DAH could be to improve feeding efficiency.

After fish larvae ingest a food item, digestion must occur to obtain energy for survival and growth. The present study indicated that grouper larvae can ingest, digest and utilize *P. similis* as an energy source, since larvae fed *P. similis* grew consistently

and survived until 10 DAH (Figs. 4, 5). The tryptic enzyme activity of the larvae, which is one of the appropriate indicators of fish larval digestion (Ueberschar, 1995; Lemieux et al., 1999; Cara et al., 2007), revealed that seven-band grouper could digest *P. similis* similarly to *B. rotundiformis* (Fig. 3).

Although the grouper larvae ingested greater numbers of *P. similis* compared with *B. rotundiformis* during the initial feeding stage (Fig. 1), larval growth was similar between the two treatments (Fig. 4). This was due to the differences in size and biomass of the two prey species. We assume that larvae fed the larger *B. rotundiformis* obtained more energy per catch than those fed the smaller *P. similis*. After 8 DAH, the selection of *B. rotundiformis* by larvae became more active (Fig. 2), resulting in better larval growth (Fig. 4). The fish larvae showed the best growth and survival (to 10 DAH) when they were co-fed the two rotifer species at the same time. In the co-feeding treatment, larvae could encounter prey items with large size variation (80-150 μm in length) and could utilize food items of appropriate size according to their growth level. The inferior growth of the larvae fed *P. similis* alone after 8 DAH was related to the lower energy content of *P. similis* in comparison to *B. rotundiformis*. These results suggest that *P. similis* can be sufficient for the growth of fish larvae, but should be fed at higher densities than *B. rotundiformis.*

EFAs for marine fish larvae, such as DHA, EPA and ARA (Watanabe et al., 1983; Izquierdo, 1996; Izquerdo et al., 2000; Lie et al., 1997; Rainuzzo et al., 1997;

Sargent et al., 1999; Takeuchi, 2001) were manipulated in *P. similis* by using an enrichment source (Table 1), as has been reported for *B. plicatilis* by many authors (Whyte et al., 1990; Tamaru et al., 1993; Kobayashi et al., 2005, 2008). The relative proportions of ARA and EPA in the total lipids of *P. similis* were higher when the *N. oculata* NIES-2146 strain was fed, while that of DHA and the ratio of DHA/EPA were higher when "super fresh" *C. vulgaris* V-12® was used. These levels of EFAs and of the DHA/EPA ratio were in the range of the suggested levels for marine fish larvae (Tucker, 1998; Sargent, 1999)

From the present study, it was clarified that the euryhaline rotifer *P. similis* can be used as a live food for rearing fish larvae, including species with small mouth gape such as groupers.

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Figure legends

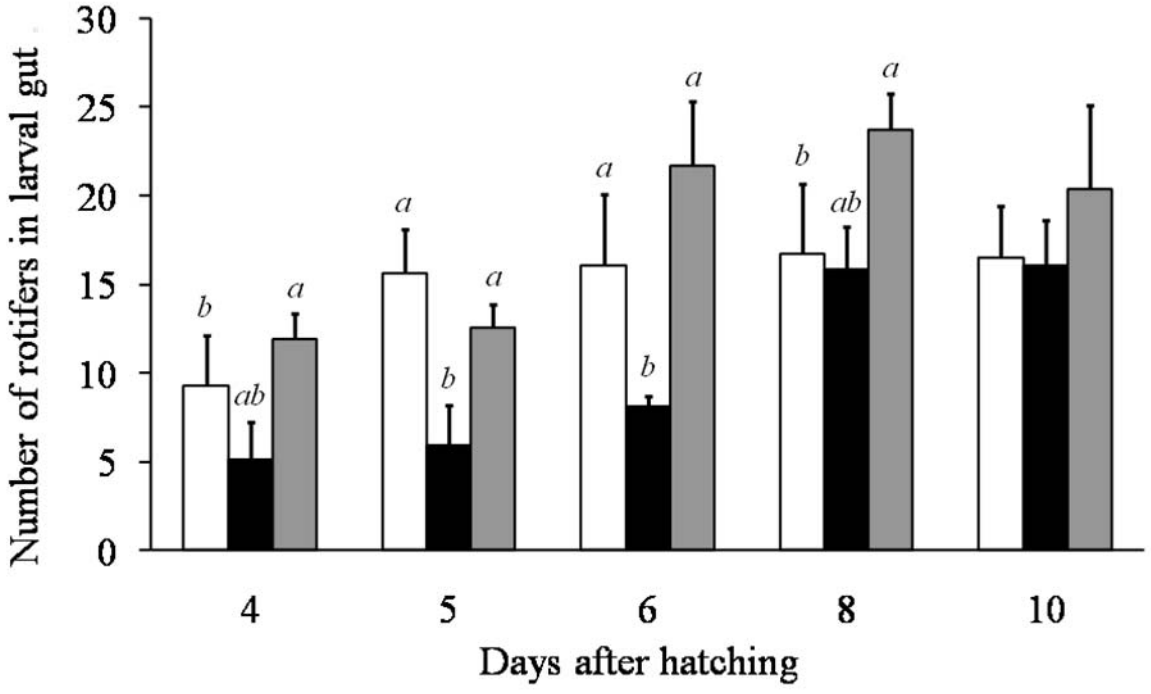

Fig. 1. Food quantity (mean±SD, n=3) of the seven-band grouper larvae fed *P. similis* at 20 ind./ml (open column), *B. rotundiformis* at 20 ind./ml (closed column) and mixed rotifers at 20 ind./ml consisting of 10 ind./ml each of *P. similis* and *B. rotundiformis* (shaded column). Different letters on the columns indicate significant differences among treatments on the same day (a>b, Tukey-Kramer test, $p<0.05$).

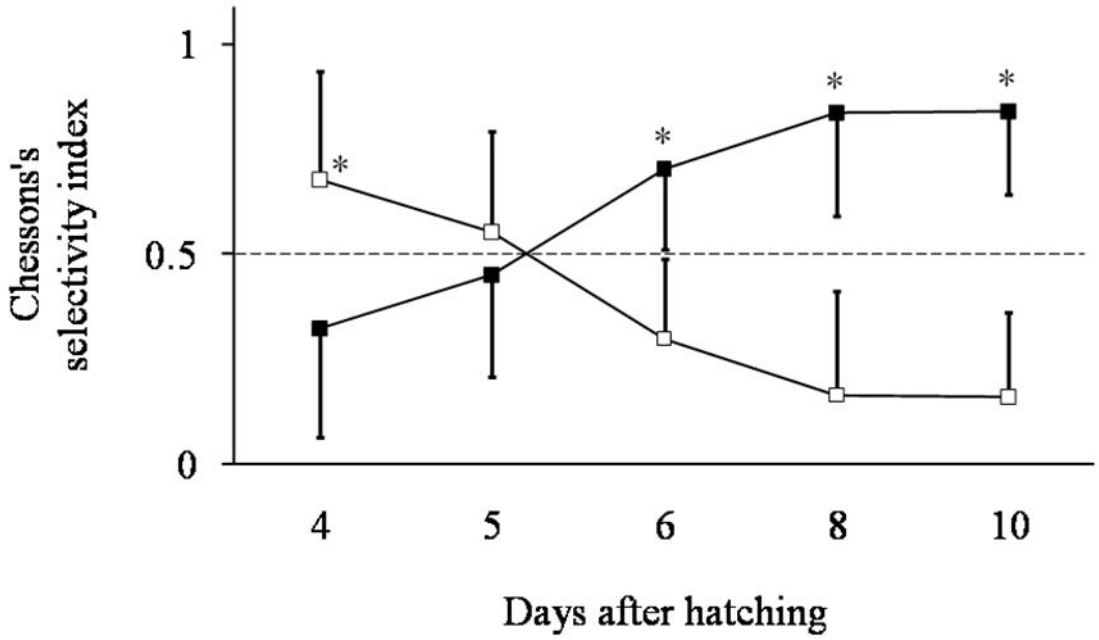

Fig. 2. Chesson's selectivity index (mean±SD, n=3) of the seven-band grouper larvae for the two rotifer species, *P. similis* (open squares) and *B. rotundiformis* (closed squares). Asterisks indicate significant differences between rotifer species at the same age (*t*-test, $p<0.05$).

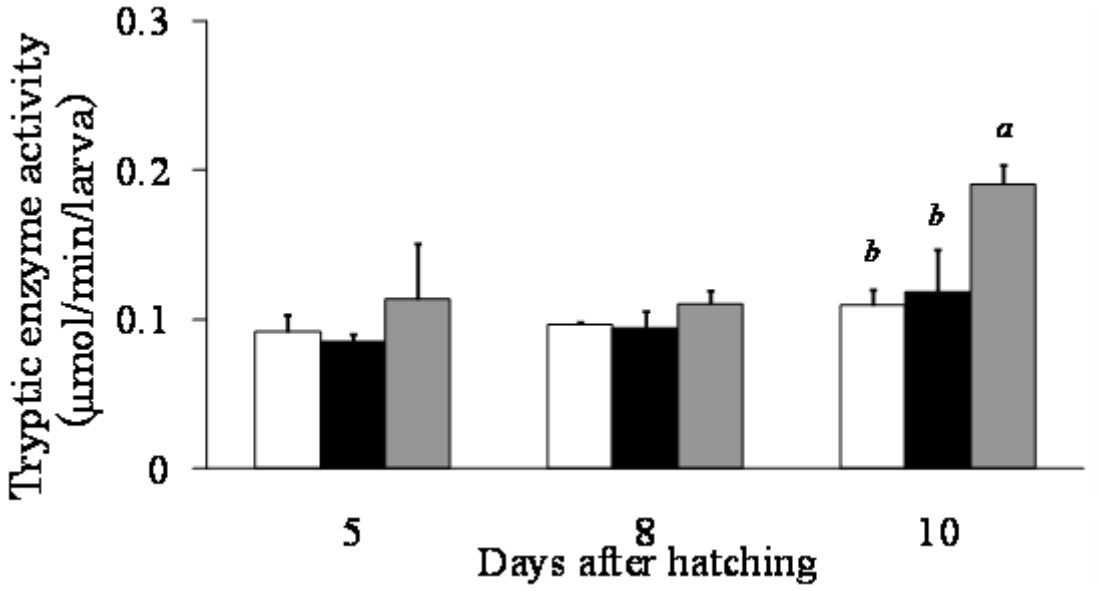

Fig. 3. Tryptic enzyme activity (mean±SD, n=3) of the seven-band grouper larvae fed *P. similis* at 20 ind./ml (open column), *B. rotundiformis* at 20 ind./ml (closed column) and mixed rotifers at 20 ind./ml consisting of 10 ind./ml each of *P. similis* and *B. rotundiformis* (shaded column). Different letters on the columns indicate significant differences among treatments at the same age (a>b, Tukey-Kramer test, $p<0.05$).

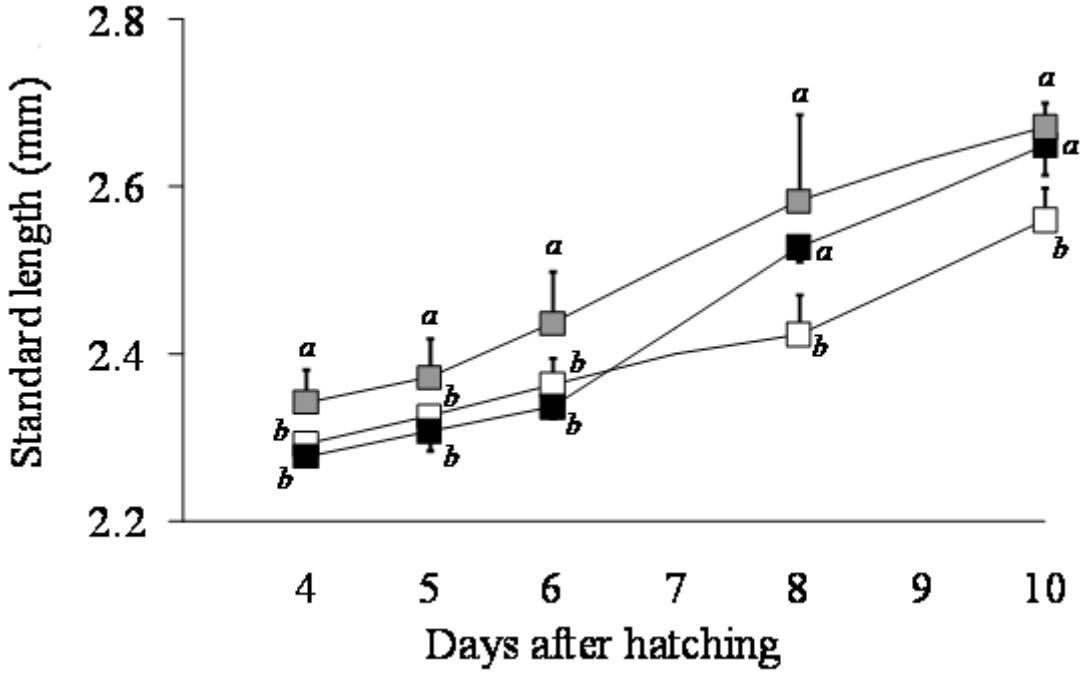

Fig. 4. Growth expressed as standard length (mean±SD, n=3) of the seven-band grouper larvae fed *P. similis* at 20 ind./ml (open square), *B. rotundiformis* at 20 ind./ml (closed square) and mixed rotifers at 20 ind./ml consisting of 10 ind./ml each of *P. similis* and *B. rotundiformis* (gray squares). Different letters on the columns indicate significant differences among treatments at the same age (a>b, Tukey-Kramer test, $p<0.05$).

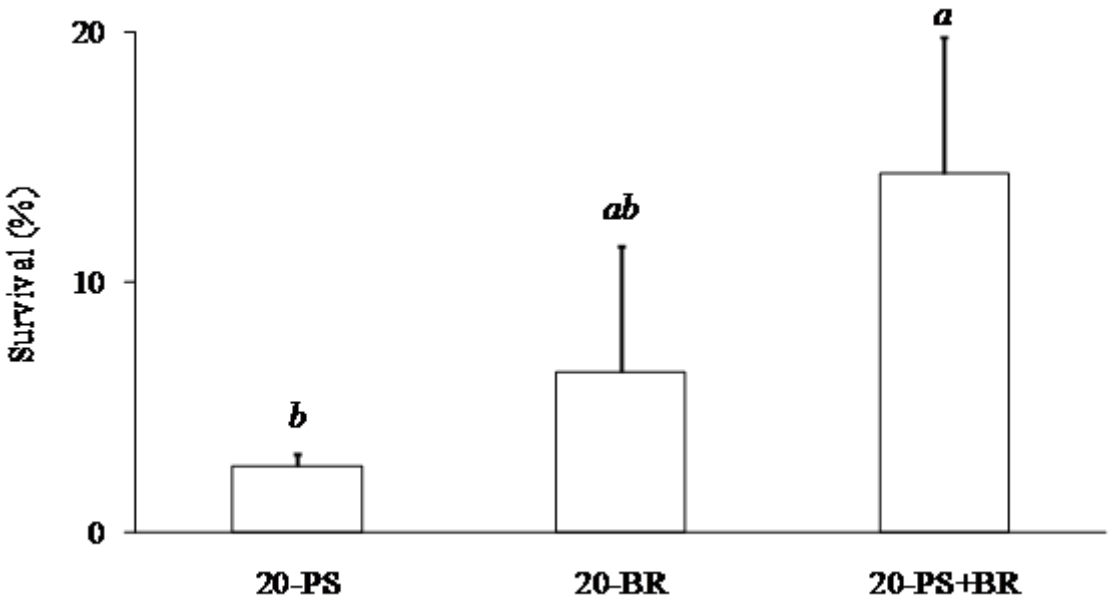

Fig. 5. Percent survival (mean±SD, n=3) at 10 DAH of the seven-band grouper larvae fed *P. similis* at 20 ind./mL (20-PS), *B. rotundiformis* at 20 ind./mL (20-BR) and mixed rotifers at 20 ind./ml consisting of 10 ind./ml each of *P. similis* and *B. rotundiformis* (20-PS+BR). Different letters on the columns indicate significant differences among treatments at the same age (a>b, Tukey-Kramer test, $p<0.05$).

Table 1. Total lipids per wet weight (%) and fatty acid composition (%) of *P. similis* fed *Nannochloropsis oculata* and "super fresh" *Chlorella vulgaris* V-12® as well as in *B. rotundiformis* fed "super fresh" *C. vulgaris* V-12®. Both rotifers were fed *C. vulgaris* V-12® prior to being fed to seven-band grouper larvae.

	Proales similis (%)		*Brachionus rotundiformis* (%)
	Nannochloropsis oculata	"Super fresh" *Chlorella vulgaris* (V-12)®	"Super fresh" *Chlorella vulgaris* (V-12)®
Total lipids	2.4	2.6	1.0
Fatty acids			
C14:0	7.6	2.6	1.7
C14:1	0.4	0.0	0.0
C16:0	18.8	11.7	18.3
C16:1	12.8	0.9	0.9
C16:2	1.1	6.0	4.7
C18:0	3.6	3.4	4.6
C18:1	9.9	6.0	2.4
C18:2 n-6	3.3	19.7	23.8
C18:3n-3	0.0	4.5	6.1
C20:0	0.3	0.3	0.3
C20:1	1.0	1.0	1.3
C20:4 n-6	5.3	0.5	1.2
C20:5 n-3	23.2	11.0	5.8
C22:0	0.4	0.5	0.3
C22:1	0.2	0.2	1.1
C22:5 n-3	2.4	3.6	3.6
C22:6 n-3	0.0	17.5	6.1
C24:0	0.8	1.3	0.4
C24:1	0.5	0.6	0.4
Others	0.5	8.7	17.0
C22:6 n-3 / C20:5 n-3	0.0	1.59	1.05
Total	100.0	100.0	100.0

Table 2. Body size and dry weight of *P. similis* and *B. rotundiformis* (mean±SD).

Species	n	Body size		Dry weight (ng/ind.;)
		Length	Width	
P. similis	50	80.7±9.0	35.4±4.0	35.9±7.8
B. rotundiformis	50	148.1±13.6	115.9±12.8	247.5±13.9