

## Effect of different algae used for enrichment of rotifers on growth, survival, and swim bladder inflation of larval amberjack *Seriola dumerili*

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**Abstract** The effect of algae with different DHA contents used for the enrichment of rotifers on the growth performance, survival, and swim bladder inflation of larval amberjack *Seriola dumerili* was investigated. Rotifers were enriched with freshwater *Chlorella vulgaris* containing three levels of DHA (rotifer containing DHA 0.04, 0.60, 1.32 g DHA 100 g<sup>-1</sup> DM) and *Nannochloropsis* (0.04 g DHA 100 g<sup>-1</sup> DM; 2.54 g EPA 100 g<sup>-1</sup>DM). The larvae were fed the enriched rotifers in triplicate from 3 days post-hatch for 7 days in static condition. The same algae used for rotifer enrichment were supplied to the larval tanks. Growth and survival rate of fish fed the rotifers enriched with *Nannochloropsis* were higher than those of fish fed the rotifers enriched with all three *Chlorella* treatments. Swim bladder inflation was lowest in fish fed the rotifers enriched with *Nannochloropsis*. The proportion of EPA was higher in fish fed the rotifers enriched with *Nannochloropsis*, while that of DHA increased proportionally with the DHA levels in the rotifers enriched with *Chlorella*. These results suggest that rotifers enriched with *Nannochloropsis* (EPA-rich rotifers) are effective to enhance growth and survival, but DHA instead of EPA is essential to improve the swim bladder inflation in amberjack larvae.

**Keywords** *Chlorella* · Enrichment · *Nannochloropsis* · Rotifer · *Seriola dumerili*

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## Introduction

An approach without water exchange during the first appropriate 10 days post-hatch has been proposed to improve the survival of marine fish larvae in mass seed production (Teruya et al. 2008, 2009). In this technique, the amount of nutrient-enriched rotifers supplied to fish larvae should be carefully adjusted and sometimes omitted because some of the previously introduced rotifers have survived and are able to reproduce in the larval-rearing tank (Yamamoto et al. 2009). To avoid the starvation of rotifers after being introduced into the larval-rearing tanks, algae such as *Chlorella* or *Nannochloropsis* used for enrichment of rotifers are added to the tanks (Yoshimatsu et al. 1995; Teruya et al. 2009). Although the nutritional value of rotifers in larval-rearing tanks without water exchange could be maintained by appropriate supplementation of algae (Yamamoto et al. 2009), the effect of the kind of algae supplied to the larval-rearing tanks on the growth and survival of larvae has not yet been clarified.

Marine fish require n-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), as essential fatty acids (EFA) for survival, normal growth, and development (Izquierdo 1996; Takeuchi 1997; Tocher 2010). Rotifers enriched with freshwater *Chlorella* (without DHA enrichment) are rich in 18:2n-6, and those with *Nannochloropsis* are particularly rich in EPA (Kobayashi et al. 2008). Therefore, freshwater *Chlorella* enriched with DHA and/or DHA-rich supplements are used for the enrichment of DHA in rotifers for seed production (Yamamoto et al. 2008). Between EPA and DHA in terms of EFA, EPA effectively improves survival as well as DHA, but does not improve fish vitality for larvae of yellowtail *Seriola quinqueradiata* (Furuuta et al. 1996b) and striped jack *Pseudocaranx dentex* (Takeuchi et al. 1996). In a recent study, DHA in rotifers has been shown to improve not only growth and survival, but swim bladder inflation of amberjack *Seriola dumerili* larvae under small-scale flow-through rearing systems (Matsunari unpubl. data). However, there is no information on the effect of EPA on amberjack larvae.

The aim of this study was to investigate the effect of rotifers enriched and fed in the larval-rearing tank without water exchange either with *Nannochloropsis* or with *Chlorella* containing three levels of DHA on the growth, survival, and swim bladder inflation of amberjack larvae.

## Materials and methods

### Rotifer enrichment and larval rearing

The feeding experiment was conducted at Shibushi Station, National Center for Stock Enhancement, Shibushi, Kagoshima, Japan, from June to July, 2009. *Brachionus rotundiformis*, so-called S-type rotifers cultured with freshwater *Chlorella* were used for the experiment. Rotifers were stocked in 200 L tanks (water volume 100 L) at a density of 500 individuals mL<sup>-1</sup> and enriched for 12 h in 100 % sea water maintained at 28 °C. Rotifers were enriched with four commercial algae: (1) freshwater *Chlorella vulgaris* (Fresh Chlorella V12, Chlorella Industry Co., Ltd., Tokyo, Japan) (treatment DHA 0), (2) DHA-enriched *C. vulgaris* (High grade Chlorella V12, HG, Chlorella Industry) (treatment DHA 0.5), (3) another DHA-enriched *C. vulgaris* (Super fresh Chlorella V12, SV, Chlorella Industry) (treatment DHA 1.0), and (4) *Nannochloropsis* (Yanmarine K-1, YK1, Chlorella Industry) (treatment Nanno). The *Chlorella* products were added at a rate of 1.0 mL L<sup>-1</sup>.

**Table 1** Foods used for rotifer enrichment and supplied to the larval-rearing tank

Treatment	<i>Chlorella</i>			<i>Nannochloropsis</i>
	DHA 0	DHA 0.5	DHA 1.0	Nanno
Food for rotifers	V12 <sup>a</sup>	HG <sup>b</sup>	SV <sup>c</sup>	YK1 <sup>d</sup>
Amount of supply				
Enrichment (mL L <sup>-1</sup> ) <sup>e</sup>	1.0	1.0	1.0	2.7
Larval tank (mL tank <sup>-1</sup> ) <sup>f</sup>	3.5	3.5	3.5	9.5

<sup>a</sup> Freshwater *Chlorella vulgaris* (Fresh Chlorella V12, Chlorella Industry Co., Ltd., Tokyo, Japan)

<sup>b</sup> DHA-enriched *Chlorella vulgaris* (High grade Chlorella V12, Chlorella Industry)

<sup>c</sup> DHA-enriched *Chlorella vulgaris* (Super fresh Chlorella V12, Chlorella Industry)

<sup>d</sup> Chilled *Nannochloropsis* (Yanmarine K-1, Chlorella Industry)

<sup>e</sup> Rotifers were enriched in a 200 L tank (water volume, 100 L)

<sup>f</sup> Larvae were reared in a 500 L tank (water volume, 500 L). Each alga was supplied twice daily

The *Nannochloropsis* were added at a rate of 2.7 mL L<sup>-1</sup>, the volume of which was the same as 1.0 mL L<sup>-1</sup> of the *Chlorella* products. The enrichment of rotifers is summarized in Table 1.

The fertilized eggs used for the experiment were obtained from reared broodstock at the Komame Branch, Stock Enhancement Technology Development Center, Otsuki, Kochi, Japan, on July 7, 2009. In the experiment, 10,000 larvae at 1 day post hatching (DPH) were stocked in each of twelve (four groups in triplicate) 500 L black polyethylene tanks (water volume 500 L) and reared until 10 DPH without water supply. At the start of the experiment, samples of fish for chemical analysis were taken from the stock tank. Aeration was provided to each tank at 0.3 L min<sup>-1</sup> through an air-stone. Photoperiod was set at 14 h light (6:00–20:00): 10 h dark. Newly enriched rotifers were added to the tanks from 3 DPH at around 8:00 and 14:00 to maintain a density of more than 10 individuals mL<sup>-1</sup>. Rotifer densities in the larval-rearing tanks were checked by sampling 5 mL of rearing water twice a day (6:00 and 13:00). To avoid starvation of rotifers after being introduced into the larval-rearing tanks, the respective *Chlorella* (3.5 mL tank<sup>-1</sup>) or *Nannochloropsis* (9.5 mL tank<sup>-1</sup>) used for the enrichment was added twice daily as food for rotifers in each larval-rearing tank. The volume of these algae was the same. A surface skimmer was installed between 3 and 6 DPH to keep the surface free from lipidic films, which is a requisite for successful swim bladder inflation (Papandroulakis et al. 2005). The total length was measured for twenty fish from each tank on 4, 6, and 10 DPH. Then, the number of rotifers in the digestive tract of larvae was counted under a stereomicroscope (SMZ1500, Nikon Corp., Tokyo, Japan) for the same twenty fish. The larvae were pressed down under a cover glass on a slide glass in order to examine their gut contents. Samples of enriched rotifers were taken three times during the experiment. The presence of air inflated swim bladder (%) was also determined using a profile projector (V-12BSC, Nikon Corp., Tokyo, Japan) for the same twenty fish from each tank on 4 and 6 DPH. The sampling of rotifers that had survived with supplemental algae in the larval-rearing tanks (tank rotifers) was done by siphoning part of the rearing water from the larval-rearing tank at the end of feeding experiment (9:00 on 11 DPH). At the end of the rearing experiment, the amberjack larvae were counted in each tank. The rotifer and fish samples were washed with freshwater, frozen immediately, and stored at -80 °C until analysis. The water temperature, dissolved oxygen (DO), and pH of the rearing water were checked twice each day during the rearing

period; mean values were 27.4–28.9 °C, 7.54–8.09 mg O<sub>2</sub> L<sup>-1</sup>, 8.11–8.45, respectively, and there were no significant differences between treatments.

### Chemical analysis

Lipids from rotifers and fish samples were extracted by the chloroform–methanol (2:1, v/v) method (Folch et al. 1957) containing 0.01 % butylhydroxytoluene. Polar and neutral lipids were separated with a silica cartridge (Sep-pak plus; Waters, Milford, MA, USA) as described by Juaneda and Rocquelin (1985). Fatty acid methyl esters (FAME) were prepared by transesterification with boron trifluoride in methanol according to the procedure of Miyashita et al. (1999) and were purified using the Sep-pak cartridge with diethyl ether/*n*-hexane (5:95). The FAME were analyzed using a gas–liquid chromatograph (GC-2010; Shimadzu, Kyoto, Japan) equipped with a hydrogen flame ionization detector and an Omegawax<sup>®</sup> 320 fused silica capillary column (30 m × 0.32 mm × 0.2 μm film thickness; Supelco, Bellefonte, PA, USA). The column temperature was initially held at 160 °C for 5 min, followed by an increase at a rate of 4 °C min<sup>-1</sup> to a final temperature of 210 °C. The carrier gas was helium, and the pressure was 80 kPa. Individual FAME were quantified with an integrator (C-R7A plus; Shimadzu) and identified by comparison with known standards (GLC-68B, Funakoshi, Tokyo, Japan and known fish oil FAME as the secondary standard) and expressed as area percent of the total identified fatty acids. Fatty acid contents in the rotifers were calculated based on the lipid content and percentage of each fatty acid in the total fatty acids.

### Statistical analysis

Data were subjected to one-way ANOVA, and the differences between treatment means were compared using Tukey's test. When the data of enriched and tank rotifers were compared, Student's *t* test was applied. For all statistical analyses, the SPSS 11.0 micro-computer software package (SPSS, Chicago, IL, USA) was used. In all statistical tests, differences at *P* < 0.05 were considered as significant.

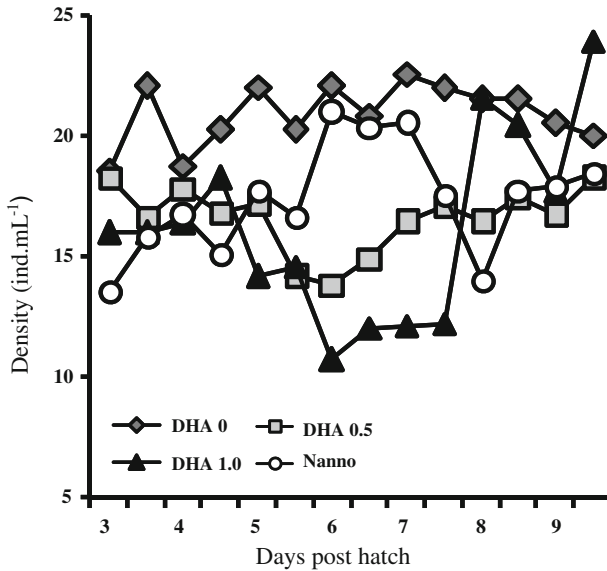
## Results

### Rotifers density in larval-rearing tank

The density of rotifers in the larval-rearing tanks during the experiment is shown in Fig. 1. The density of rotifers in treatment DHA 0, 0.5, and Nanno in the rearing tank fluctuated between 12 and 22 rotifers mL<sup>-1</sup>. Since the density of rotifers in treatment DHA 1.0 decreased from 17 to 11 rotifers mL<sup>-1</sup> around 5 DPH, newly enriched rotifers were added to the tanks to maintain a density of more than 10 rotifers mL<sup>-1</sup> at 6, 7, and 8 DPH. To the other treatment groups, newly enriched rotifers were not added.

### Lipid contents and fatty acid composition of enriched rotifers

The lipid contents and certain fatty acid compositions of the enriched rotifers are shown in Table 2. There were no significant differences in the lipid contents among the treatments. The proportions of linoleic acid (LA; 18:2n-6) and linolenic acid (LNA; 18:3n-3) were most abundant in the rotifers of treatment DHA 0. The proportion of EPA in the total lipids



**Fig. 1** Rotifer density in the larval-rearing tanks ( $n = 3$ )

**Table 2** Crude lipid content and proportion of major fatty acids in enriched rotifers used for the feeding trial

	<i>Chlorella</i>			<i>Nannochloropsis</i>
	DHA 0	DHA 0.5	DHA 1.0	
Crude lipid (g 100 g <sup>-1</sup> , DM)	13.8 ± 0.5 <sup>a</sup>	14.6 ± 0.7 <sup>a</sup>	15.3 ± 0.4 <sup>a</sup>	14.8 ± 1.0 <sup>a</sup>
Fatty acid (area, %)*				
18:1	2.6 ± 0.1 <sup>a</sup>	2.6 ± 0.1 <sup>a</sup>	2.6 ± 0.2 <sup>a</sup>	3.8 ± 0.2 <sup>b</sup>
18:2n-6	31.0 ± 1.7 <sup>c</sup>	27.9 ± 1.7 <sup>bc</sup>	23.9 ± 1.8 <sup>b</sup>	21.9 ± 2.7 <sup>a</sup>
18:3n-3	10.8 ± 0.9 <sup>c</sup>	9.3 ± 0.8 <sup>bc</sup>	7.9 ± 0.7 <sup>b</sup>	2.3 ± 0.8 <sup>a</sup>
20:4n-6	0.2 ± 0.1 <sup>a</sup>	0.3 ± 0.1 <sup>a</sup>	0.4 ± 0.1 <sup>a</sup>	3.6 ± 0.4 <sup>b</sup>
20:5n-3	0.2 ± 0.0 <sup>a</sup>	2.5 ± 0.1 <sup>ab</sup>	4.5 ± 0.3 <sup>b</sup>	17.3 ± 3.2 <sup>c</sup>
22:5n-3	0.1 ± 0.0 <sup>a</sup>	0.9 ± 0.1 <sup>b</sup>	1.9 ± 0.2 <sup>c</sup>	3.0 ± 0.5 <sup>d</sup>
22:6n-3	0.3 ± 0.0 <sup>a</sup>	4.1 ± 0.2 <sup>b</sup>	8.6 ± 0.9 <sup>c</sup>	0.3 ± 0.0 <sup>a</sup>
∑n-3 LC-PUFA	2.4 ± 0.3 <sup>a</sup>	9.2 ± 0.5 <sup>b</sup>	16.7 ± 1.4 <sup>c</sup>	21.5 ± 3.2 <sup>d</sup>
EPA (g 100 g <sup>-1</sup> , DM)	0.03 ± 0.00 <sup>a</sup>	0.36 ± 0.00 <sup>b</sup>	0.69 ± 0.03 <sup>c</sup>	2.54 ± 0.37 <sup>d</sup>
DHA (g 100 g <sup>-1</sup> , DM)	0.04 ± 0.00 <sup>a</sup>	0.60 ± 0.01 <sup>b</sup>	1.32 ± 0.10 <sup>c</sup>	0.04 ± 0.01 <sup>a</sup>
∑n-3 LC-PUFA (g 100 g <sup>-1</sup> , DM)	0.33 ± 0.04 <sup>a</sup>	1.34 ± 0.04 <sup>a</sup>	2.54 ± 0.15 <sup>b</sup>	3.16 ± 0.40 <sup>c</sup>
DHA/EPA	1.38 ± 0.14 <sup>b</sup>	1.67 ± 0.02 <sup>c</sup>	1.91 ± 0.06 <sup>d</sup>	0.02 ± 0.01 <sup>a</sup>

Mean ± SD,  $n = 3$

Values with the same superscript letter within the same row in each treatment are not significantly different ( $P > 0.05$ )

\* Percentage of total fatty acid

was highest in the rotifers of treatment Nanno, intermediate in rotifers of treatments DHA 0.5 and 1.0, and lowest in rotifers of treatment DHA 0. The proportions of DHA in the total lipids of rotifers of treatment DHA 0.5 and 1.0 were significantly higher compared to the rotifers of treatment DHA 0 and Nanno. The DHA/EPA of rotifers increased from 1.38 (DHA 0) to 1.91 (DHA 1.0), while the DHA/EPA of rotifers enriched with *Nannochloropsis* was 0.02.

#### Lipid contents and fatty acid composition of rotifers in larval-rearing tanks

The lipid contents and certain fatty acid compositions of tank rotifers are shown in Table 3. The total lipid content of tank rotifers in treatment Nanno was significantly higher compared with the tank rotifers enriched with the three *Chlorella* products. The proportions LA and LNA in the total lipids of rotifers of treatment DHA 0 were most abundant. The proportion of EPA and n-3 LC-PUFA in the total lipids of rotifers was highest in treatment Nanno. The proportion of DHA in the total lipids was highest in the rotifers of treatment DHA 1.0, intermediate in rotifers of treatments DHA 0.5 and 0, and lowest in rotifers of treatment Nanno. Compared to the total lipid contents of the enriched rotifers, the contents of the tank rotifers significantly decreased ( $P < 0.05$ ) (Fig. 2). The proportions of EPA in the tank rotifers were significantly higher relative to the enriched ones, except for treatment Nanno. The proportions of DHA of rotifers in treatment DHA 0.5, 1.0, and Nanno were not significantly different between the enriched and tank rotifers, while the proportion of tank rotifers of treatment DHA 0 was significantly higher compared to the enriched ones. The DHA/EPA of tank rotifers in the *Chlorella*-treated groups was approximately 1.0, while the DHA/EPA of tank rotifer fed with *Nannochloropsis* was 0.01.

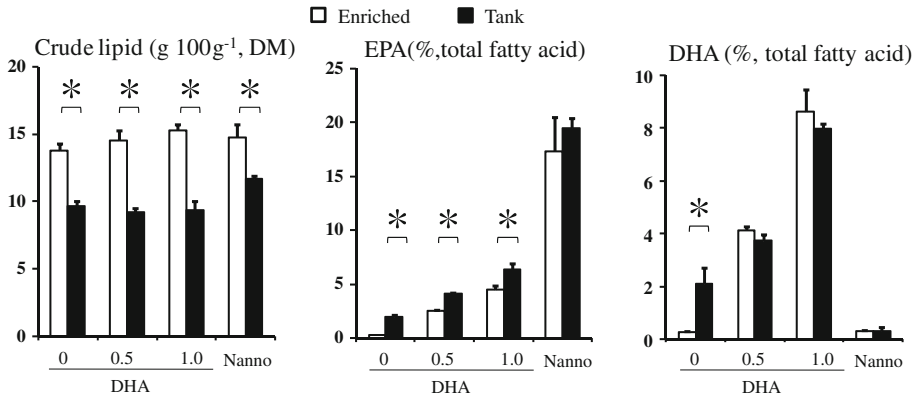
**Table 3** Crude lipid content and proportion of major fatty acids in tank rotifers used for the feeding trial

	<i>Chlorella</i>			<i>Nannochloropsis</i>
	DHA 0	DHA 0.5	DHA 1.0	
Crude lipid (g 100 g <sup>-1</sup> , DM)	9.7 ± 0.4 <sup>a</sup>	9.2 ± 0.3 <sup>a</sup>	9.4 ± 0.7 <sup>a</sup>	11.7 ± 0.3 <sup>b</sup>
Fatty acid (area, %)*				
18:1	4.5 ± 0.7 <sup>a</sup>	5.0 ± 0.2 <sup>a</sup>	4.9 ± 0.1 <sup>a</sup>	5.0 ± 0.0 <sup>a</sup>
18:2n-6	26.9 ± 2.6 <sup>c</sup>	26.5 ± 0.2 <sup>c</sup>	20.6 ± 0.6 <sup>b</sup>	6.6 ± 0.1 <sup>a</sup>
18:3n-3	6.9 ± 0.7 <sup>d</sup>	5.7 ± 0.2 <sup>c</sup>	4.7 ± 0.2 <sup>b</sup>	0.3 ± 0.0 <sup>a</sup>
20:4n-6	0.9 ± 0.1 <sup>a</sup>	0.6 ± 0.1 <sup>a</sup>	0.7 ± 0.1 <sup>a</sup>	5.3 ± 1.1 <sup>b</sup>
20:5n-3	1.9 ± 0.2 <sup>a</sup>	4.0 ± 0.1 <sup>b</sup>	6.3 ± 0.6 <sup>c</sup>	19.5 ± 0.9 <sup>d</sup>
22:5n-3	0.9 ± 0.5 <sup>a</sup>	1.7 ± 0.0 <sup>a</sup>	3.4 ± 0.1 <sup>c</sup>	6.0 ± 0.7 <sup>d</sup>
22:6n-3	2.1 ± 0.6 <sup>b</sup>	3.7 ± 0.2 <sup>c</sup>	8.0 ± 0.2 <sup>d</sup>	0.3 ± 0.2 <sup>a</sup>
∑n-3 LC-PUFA	6.1 ± 1.2 <sup>a</sup>	10.8 ± 0.3 <sup>b</sup>	18.6 ± 0.7 <sup>c</sup>	26.7 ± 1.1 <sup>d</sup>
EPA (g 100 g <sup>-1</sup> , DM)	0.18 ± 0.02 <sup>a</sup>	0.37 ± 0.01 <sup>b</sup>	0.59 ± 0.07 <sup>c</sup>	2.28 ± 0.14 <sup>d</sup>
DHA (g 100 g <sup>-1</sup> , DM)	0.20 ± 0.06 <sup>b</sup>	0.34 ± 0.03 <sup>b</sup>	0.75 ± 0.06 <sup>c</sup>	0.03 ± 0.02 <sup>a</sup>
∑n-3 LC-PUFA (g 100 g <sup>-1</sup> , DM)	0.58 ± 0.11 <sup>a</sup>	0.99 ± 0.04 <sup>a</sup>	1.75 ± 0.15 <sup>b</sup>	3.12 ± 0.18 <sup>c</sup>
DHA/EPA	1.07 ± 0.20 <sup>bc</sup>	0.92 ± 0.06 <sup>b</sup>	1.27 ± 0.09 <sup>c</sup>	0.01 ± 0.01 <sup>a</sup>

Mean ± SD,  $n = 3$

Values with the same superscript letter within the same row in each treatment are not significantly different ( $P > 0.05$ )

\* Percentage of total fatty acid



**Fig. 2** Crude lipid and the proportions of EPA and DHA in the enriched rotifers (white) and the tank rotifers (black). Values are mean  $\pm$  SD ( $n = 3$ ). An asterisk means a significant difference ( $P < 0.05$ ) between the enriched rotifers and the tank rotifers

Growth, feeding, survival, and swim bladder inflation of amberjack larvae

The growth of fish was not significantly different between the treatments (Table 4). There were no significant differences in the number of rotifers found in the digestive tract among the treatment, but the value at 10 DPH of fish in treatment DHA 0 was lower, although not significantly, compared to values of fish fed the other rotifers (Fig. 3). The survival rate of fish fed the rotifers enriched with *Nannochloropsis* was significantly higher than that of fish fed the rotifers enriched with the *Chlorella* products. Among the rotifers enriched with the *Chlorella* products, the survival rates of fish fed the rotifers in treatment DHA 0.5 and 1.0 tended to be improved compared to the rate in treatment DHA 0, although significant differences were not found among them. The swim bladder inflation of fish was not significantly different between the treatments, but the value was numerically highest in treatment DHA 0.5, followed by treatment DHA 1.0, DHA 0, and Nanno.

**Table 4** Effect of feeding rotifers enriched with *Chlorella* or *Nannochloropsis* on growth, survival rate, and swim bladder inflation of amberjack larvae

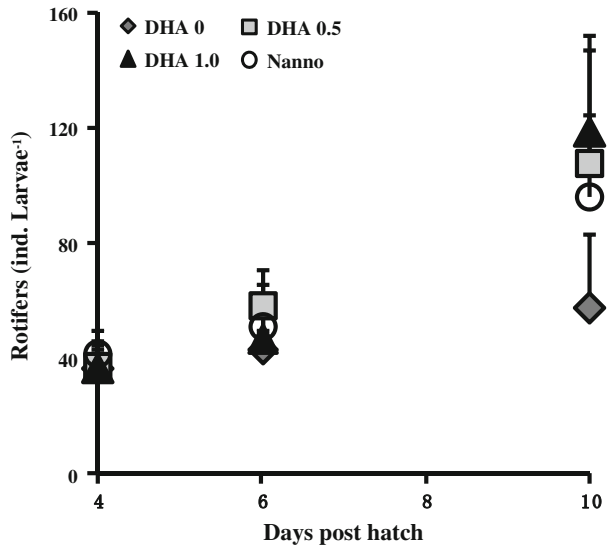
	<i>Chlorella</i>			<i>Nannochloropsis</i>
	DHA 0	DHA 0.5	DHA 1.0	
Initial				
Total length (mm)	3.7 $\pm$ 0.0 <sup>a</sup>	3.7 $\pm$ 0.0 <sup>a</sup>	3.7 $\pm$ 0.0 <sup>a</sup>	3.7 $\pm$ 0.0 <sup>a</sup>
Final				
Total length (mm)	4.7 $\pm$ 0.1 <sup>a</sup>	4.9 $\pm$ 0.1 <sup>a</sup>	4.8 $\pm$ 0.1 <sup>a</sup>	5.0 $\pm$ 0.1 <sup>a</sup>
Survival rate (%)	4.1 $\pm$ 2.6 <sup>a</sup>	16.0 $\pm$ 2.5 <sup>a</sup>	14.8 $\pm$ 9.9 <sup>a</sup>	37.6 $\pm$ 3.8 <sup>b</sup>
Swim bladder inflation (%) <sup>*</sup>	23.3 $\pm$ 9.5 <sup>a</sup>	39.2 $\pm$ 10.4 <sup>a</sup>	31.7 $\pm$ 29.3 <sup>a</sup>	14.2 $\pm$ 11.8 <sup>a</sup>

Mean  $\pm$  SD,  $n = 3$

Values with the same superscript letter within the same row in each treatment are not significantly different ( $P > 0.05$ )

<sup>\*</sup> Values given are the means of 4 and 6 DPH

**Fig. 3** Changes in the number of rotifers in digestive tract of amberjack larvae. Data are expressed as the mean + SD ( $n = 3$ ) of three replicate tanks. There were no significant differences in the number of rotifers in digestive tract among the treatment



Lipid contents and fatty acid composition of larvae

The lipid contents and compositions of certain fatty acids of the polar lipids of larvae are shown in Table 4. There were no significant differences in the total lipid contents among the treatments. In fish of treatment Nanno, the proportion of EPA in the polar lipid fraction was significantly higher, and that of DHA was significantly lower than those of fish in the *Chlorella*-treatment groups. The levels of DHA in the polar lipid fraction increased proportionally with the DHA levels of the tank rotifers (Table 5).

**Table 5** Crude lipid content and proportion of major fatty acids of polar lipid in amberjack larvae whole body

	Initial	Final			
		<i>Chlorella</i>			<i>Nannochloropsis</i>
		DHA 0	DHA 0.5	DHA 1.0	
Crude lipid (g 100 g <sup>-1</sup> , DM)	20.3	15.5 ± 0.5 <sup>a</sup>	12.8 ± 0.7 <sup>a</sup>	13.8 ± 1.7 <sup>a</sup>	13.3 ± 1.0 <sup>a</sup>
Fatty acid (area, %)*					
18:1	10.0	7.4 ± 0.3 <sup>b</sup>	6.4 ± 0.1 <sup>a</sup>	5.9 ± 0.3 <sup>a</sup>	8.4 ± 0.4 <sup>a</sup>
18:2n-6	0.9	24.6 ± 1.1 <sup>d</sup>	18.8 ± 1.0 <sup>c</sup>	12.8 ± 0.1 <sup>b</sup>	3.5 ± 0.2 <sup>a</sup>
18:3n-3	0.3	3.4 ± 0.3 <sup>c</sup>	1.7 ± 0.2 <sup>b</sup>	1.2 ± 0.1 <sup>b</sup>	0.1 ± 0.0 <sup>a</sup>
20:4n-6	2.7	2.4 ± 0.1 <sup>b</sup>	2.0 ± 0.2 <sup>ab</sup>	1.6 ± 0.0 <sup>a</sup>	7.6 ± 0.3 <sup>c</sup>
20:5n-3	7.3	0.9 ± 0.2 <sup>a</sup>	4.8 ± 0.1 <sup>b</sup>	4.9 ± 0.1 <sup>c</sup>	12.2 ± 0.5 <sup>d</sup>
22:5n-3	1.6	0.6 ± 0.1 <sup>a</sup>	3.2 ± 0.0 <sup>b</sup>	4.2 ± 0.3 <sup>c</sup>	11.8 ± 0.6 <sup>d</sup>
22:6n-3	28.2	11.5 ± 0.3 <sup>b</sup>	14.9 ± 0.3 <sup>c</sup>	22.1 ± 0.7 <sup>d</sup>	4.2 ± 0.1 <sup>a</sup>
∑n-3 LC-PUFA	37.4	15.6 ± 0.2 <sup>a</sup>	23.6 ± 0.2 <sup>b</sup>	31.9 ± 0.4 <sup>d</sup>	28.4 ± 1.0 <sup>c</sup>

Mean ± SD,  $n = 3$

Values with the same superscript letter within the same row in each treatment are not significantly different ( $P > 0.05$ )

\* Percentage of total fatty acid



## Discussion

In the present study, the rotifer density in the larval-rearing tanks of treatment DHA 1.0 decreased around 5 DPH. The productivity of rotifers varies according to the environmental conditions such as temperature and DO level. Low levels of DO ( $<0.8 \text{ mg L}^{-1}$ ) and sudden drop of DO (from  $6.1$  to  $2.3 \text{ mg L}^{-1}$ ) have been reported to induce impaired productivity of rotifers (Yamasaki et al. 1987; Koiso and Hino 2006). In the present study, DO was more than  $6.0 \text{ mg L}^{-1}$  and a sudden drop of DO was not observed. On the other hand, the density of rotifers in the larval-rearing tanks of treatment DHA 0, 0.5, and Nanno were maintained between 12 and  $22 \text{ ind. mL}^{-1}$ . The low productivity of rotifers observed in treatment DHA 1.0 may be due to the higher levels of DHA in the *Chlorella*. In the rotifer of treatment DHA 1.0, not only the density but also the activity (swimming frequency of rotifers) decreased. In many cases in Japan, DHA-enriched fresh water *Chlorella* is supplied to the larval-rearing tank for preventing the rotifers in the tank from starvation (Yoseda et al. 2008; Hamasaki et al. 2009). There are several reports dealing with the nutritional value of rotifers enriched with different algae (Watanabe et al. 1983; Reitan et al. 1993). However, the effect of the kind of algae on the activity and productivity of rotifers has rarely been assessed. Detailed investigations of the different algae used for enrichment of rotifers on activity and productivity of rotifers are necessary.

Feeding density of rotifers is suggested to affect the growth performance in certain fish species. In bluefin tuna *Thunnus thynnus* larvae, the optimal rotifer density in the tank was estimated to be from 10 to  $30 \text{ ind. mL}^{-1}$  for the initial feeding (Sawada et al. 2000). In grouper *Epinephelus suillus* larvae, rotifer intake and growth of fish fed rotifers at differing densities (5, 10, and  $20 \text{ ind. mL}^{-1}$ ) were not significantly different between the treatments (Duray et al. 1996). In the present study, the density of rotifers in the larval-rearing tanks of all treatment was maintained from 10 to  $25 \text{ ind. mL}^{-1}$ . In addition, there were no significant differences in the number of rotifers in the digestive tract among the treatment. Thus, the differences in the density of rotifers have no impact on larval performance.

The nutritional value of rotifers in larval-rearing tanks without water exchange can be maintained by appropriate supplementation of algae (Yamamoto et al. 2009). This observation was reconfirmed in the present study; the proportions of EPA and DHA of the tank rotifers reflect the proportions of the algae (*Nannochloropsis* and DHA-enriched *Chlorella*) supplemented to the larval-rearing tanks. On the other hand, the proportions of EPA and DHA of rotifers in treatment DHA 0 significantly increased compared to the enriched ones, although the *Chlorella* contains no EPA and DHA (Kobayashi et al. 2008). A similar trend was also observed in the continuous-culture rotifers; the proportion of DHA of rotifers fed DHA-unenriched *Chlorella* in the harvest tank increased relative to that in the cultivation tank (Kotani et al. 2009). Rotifers have low synthetic capability of LC-PUFA from precursor molecules. Rotifers fed on baker's yeast for several generations contained LC-PUFA, although the yeast contained mainly palmitoleic acid and oleic acid and was completely devoid of LC-PUFA (Lubzens et al. 1985). Thus, the increase in EPA and DHA levels of rotifers fed the fatty acids-unenriched *Chlorella* in larval-rearing tank in the present study is probably attributable to a result of LC-PUFA synthesis and elongation from the precursors by the rotifers.

The highest growth and survival rates were observed in amberjack larvae fed rotifers of treatment Nanno (EPA-rich rotifers), compared to larvae fed rotifers fed with other *Chlorella* products. An increase in dietary n-3 LC-PUFA level improved the larval growth of gilthead sea bream *Sparus aurata* (Rodríguez et al. 1994) and Japanese flounder *Paralichthys olivaceus* (Izquierdo et al. 1992; Furuita et al. 1999). The contents of n-3 LC-

PUFA in the tank rotifers treated with *Nannochloropsis* were  $3.12 \text{ g } 100 \text{ g}^{-1}$  (EPA  $2.28 \text{ g } 100 \text{ g}^{-1}$ ), while those with the three *Chlorella* products were from  $0.58$  to  $1.75 \text{ g } 100 \text{ g}^{-1}$  dry matter basis (DM). The optimum n-3 LC-PUFA level in rotifers for maximum survival rate in amberjack larvae reared in a flow-through system have been estimated to be approximately  $2.5 \text{ g } 100 \text{ g}^{-1}$  DM (EPA  $0.5 \text{ g } 100 \text{ g}^{-1}$ , DHA  $1.5 \text{ g } 100 \text{ g}^{-1}$ ) (Matsunari unpubl. data). Thus, EPA effectively improved growth and survival as well as DHA in amberjack larvae as shown in other marine fish larvae such as red sea bream *Pagrus major* (Watanabe et al. 1989), yellowtail (Furuita et al. 1996b), and striped jack (Takeuchi et al. 1996). The requirement of n-3 LC-PUFA is determined not only as its absolute amount in the diet (food), but also as the relative proportion of DHA and EPA. The ratio of dietary DHA to EPA has been suggested to affect the normal growth and development in certain fish species (Watanabe and Kiron 1994; Rodríguez et al. 1997). In the present study, there was no correlation between the DHA/EPA and growth or survival rate of larval amberjack. However, there was a large divergence with the proportions of EPA, DHA, and n-3 LC-PUFA of rotifers in each treatment. Therefore, the ratio of dietary DHA/EPA was not used for evaluating the nutritional value of EFA in rotifers enriched with *Nannochloropsis*.

In seed production, two different phenomena have been indicated as the causes of mass mortality (Miyashita 2006). The first phenomenon is the adhesion of larvae to the water surface. The other is the larval contact with the tank bottom due to their weak upward swimming ability against the gravitational force and infrequent swimming behavior during the night-time (Papandroulakis et al. 2005; Takashi et al. 2006). Mortality due to the latter phenomenon has been associated with the lack of a functional swim bladder (Kitajima et al. 1993). Initial swim bladder inflation is achieved via ingesting air at the water surface during a brief but finite period, when the pneumatic duct connecting the gut and the swim bladder is open (Rieger and Summerfelt 1998). In larval amberjack, swim bladder inflation has been suggested to be achieved in a similar way (Teruya et al. 2009). In the present study, the swim bladder inflation of fish fed rotifers treated with *Nannochloropsis*, although not statistically different from the others, was inferior to that of other fish fed rotifers treated with *Chlorella*. The n-3 LC-PUFA level in rotifers for improvement of swim bladder inflation in amberjack larvae reared in a flow-through system have been estimated to be approximately  $2.0 \text{ g } 100 \text{ g}^{-1}$  DM (Matsunari unpubl. data). Although the content of n-3 LC-PUFA in the tank rotifers treated with *Nannochloropsis* was  $3.12 \text{ g } 100 \text{ g}^{-1}$  DM, the swim bladder inflation was not improved in amberjack larvae fed rotifers of treatment Nanno. On the other hand, the DHA level of tank rotifers in the *Chlorella*-treated groups was from  $0.20$  to  $0.75$ , while that of the rotifers enriched with *Nannochloropsis* was  $0.03$ . The swim bladder inflation of amberjack larvae fed rotifers enriched with DHA was superior to that of fish fed rotifers without DHA enrichment (Matsunari unpubl. data). In terms of DHA, the rotifers of treatment Nanno would not be sufficient to satisfy the requirement for improvement of swim bladder inflation. In larval red sea bream and yellowtail, EPA is inferior to DHA as an EFA from the viewpoint of vitality (Watanabe et al. 1989; Furuita et al. 1996a, b). Thus, the lower swim bladder inflation of amberjack larvae fed rotifers with *Nannochloropsis* (rotifers contain little DHA) could be attributable to the dietary deficiency of DHA, resulting in having difficulties in remaining beneath the surface or penetrating the water surface due to their poorer vitality.

The results of the present study suggest that the rotifers enriched with *Nannochloropsis* (EPA-rich rotifers) are effective for enhancing growth and survival, but are not able to improve the swim bladder inflation in amberjack larvae during the post-hatch period under static water conditions.

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