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ORIGINAL ARTICLE

Aquaculture

Nutritional evaluation of rotifers in rearing tanks without water exchange during seed production of amberjack *Seriola dumerili*

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Abstract We evaluated the nutrient contents of rotifers sampled from larval-rearing tanks (tank rotifers) without water exchange during the seed production of amberjack *Seriola dumerili* at three facilities (Kamiura, Kagoshima, and Miyazaki) and compared them with the nutrient contents of freshly enriched rotifers. Compared to the enriched rotifers, the lipid contents, especially neutral lipids and proportion of 22:6n-3, tended to decrease in the tank rotifers. These trends were clearer at Miyazaki where the tank rotifers were sampled before daily supplementation of microalgae (*Nannochloropsis*). Crude protein content of the tank rotifers did not decrease markedly although the proportion of lysine tended to decrease. Vitamin C and E contents of the tank rotifers decreased significantly only at Miyazaki. Calcium content of the tank rotifers increased at Kamiura and Miyazaki, and the increases in iron and manganese contents of the tank rotifers at Miyazaki and zinc content at Kagoshima were pronounced. These results suggest that the nutritional value of rotifers in larval-rearing tanks without water exchange can be maintained by appropriate supplementation of microalgae. The effect of certain minerals that became high in tank rotifers on subsequent larval development requires further investigation.

Keywords Amino acid · Fatty acid · Mineral · Rotifer · *Seriola dumerili* · Vitamin

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Introduction

To improve the survival of marine fish larvae during mass seed production, an approach with no or a low water exchange has been proposed [1–4]. The effectiveness of the approach without any water exchange during the first 5 to 10 days after hatching has recently been verified in the seed production of groupers [5, 6] and amberjack *Seriola dumerili* (http://www.affrc.go.jp/ja/research/seika/data_suisan/h17/nria/nria004, in Japanese). In this new rearing technique, the amount of nutrient-enriched rotifers supplied to fish larvae is adjusted every day and sometimes omitted because some of the previously introduced rotifers have survived and are able to reproduce in the larval-rearing tank. To prevent the starvation of the rotifers in the larval-rearing tank, microalgae such as *Chlorella* or *Nannochloropsis* are usually supplied to the tank [1, 2, 5, 6]. Thus, fish larvae feed on both the freshly enriched rotifers as well as the rotifers that have survived and fed on the

microalgae in the larval-rearing tank. Although there are several reports dealing with the nutritional value of starved rotifers [1, 2, 4, 7–9] and algal-supplemented rotifers under laboratory stagnant conditions without fish larvae [1, 2], the extent to which the nutritional value of the rotifers is maintained in the larval-rearing tank without water exchange remains to be ascertained.

In this study, we examined the nutrient compositions of freshly enriched rotifers (enriched rotifers) and also rotifers that had survived with supplemental microalgae in the larval-rearing tanks (tank rotifers) for mass seed production of amberjack. The rotifer samples were obtained during the post-hatch periods without water supply from three facilities where amberjack seeds are produced.

Materials and methods

Sample collection

Enriched rotifers and tank rotifers from the larval-rearing tanks for mass seed production of amberjack were sampled at the Stock Enhancement Technology Development Center (Kamiura), Saiki, Oita, Japan; the Kagoshima Prefectural Fisheries Technology and Development Center (Kagoshima), Ibusuki, Kagoshima, Japan; and the Miyazaki Fisheries Promotion Association (Miyazaki), Nobeoka, Miyazaki, Japan. The samples were obtained from two production trials at each facility.

During the periods without water supply to the larval-rearing tanks, both the S-type rotifer *Brachionus rotundiformis* and the L-type rotifer *B. plicatilis* were used in the second trial at Kamiura and both trials at Kagoshima, while only S-type rotifers were used in the first trial at Kamiura and both trials at Miyazaki. The enriched rotifers were sampled from their culture tank at the same time as they were supplied to the larval tank, two or three times in each production trial. The rotifers were cultured with 22:6n-3 (DHA)-enriched *Chlorella vulgaris* (Super Fresh Chlorella V12, SV12, Chlorella Industry, Tokyo, Japan) at Kamiura and Kagoshima. The rotifers provided to fish in the afternoon at Kagoshima were further enriched with a commercial DHA emulsion (Marine Glos, Nisshin Marine Tech, Yokohama, Japan) during the morning. The S-type rotifers at Miyazaki were enriched with *Nannochloropsis* sp. and a commercial DHA emulsion (Plus AQUARAN, BASF Japan, Tokyo, Japan). To avoid the starvation of rotifers after their introduction into the larval-rearing tank, SV12 (at Kamiura) or *Nannochloropsis* (at Kagoshima and Miyazaki) were added to the rearing tank every day. The enrichment of rotifers and supplementation of the microalgae to the larval tanks are summarized in Table 1. The sampling of tank rotifers from the larval-rearing tank was conducted before freshly enriched rotifers of the day were supplied, twice in each production trial, by siphoning part of the rearing water. All rotifer samples were immediately stored at –20°C for chemical analyses.

Analytical method

Determinations of moisture and crude protein content of the rotifer samples were made by drying samples for 10 h at 110°C and by using the semimicro Kjeldahl method (N × 6.25), respectively. The samples were also extracted with a mixture of chloroform and methanol containing 0.01% butylhydroxytoluene for determination of the total lipid content [10]. The extracted lipids were then separated into neutral and polar lipids with a silica cartridge (SepPak Plus, Waters, Milford, MA, USA) [11]. The preparation of fatty acid methyl esters and the conditions for fatty acid composition analysis by gas liquid chromatography (GC-2010, Shimadzu, Kyoto, Japan) were the same as described previously [12]. Free amino acid compositions of the samples were determined by an automatic amino acid analyzer L-8500 (Hitachi, Tokyo, Japan) after the samples were extracted in 0.6 N perchloric acid. The total amino acid compositions of the samples were determined using an automatic amino acid analyzer L-8800 (Hitachi) after the samples were hydrolyzed in 6 N HCl for 22 h at 110°C. The tryptophan and cystine contents were not determined due to limited amounts of the samples. Mineral contents were measured by an inductively coupled spectrometer (ICP) (Leeman Laboratories, Lowell, MA, USA) after the samples were hydrolyzed in nitric acid using a microwave oven ETHOS-D (Milestone General, Kawasaki, Japan). Vitamin C contents of the samples were determined by a high performance liquid chromatography (HPLC) (Shimadzu) according to the method of Koshio et al. [13], using L-ascorbic acid as the standard. The vitamin E contents of the samples were also determined by HPLC according to the method of Furuita et al. [12], using DL-α-tocopherol as the standard. Analyses of certain samples for crude protein, total amino acids (hydrolyzed), vitamins, and minerals were not carried out due to limited amounts of the samples.

Data between the enriched rotifers and the tank rotifers were compared by one-way ANOVA, and a probability level of less than 0.05 was considered as significant. The data are expressed as mean ± SD on a dry-matter basis.

Results

The supplementation of enriched rotifers to the larval-rearing tanks and the sampling of the tank rotifers in each trial at three facilities are summarized in Table 2. The tank rotifers for chemical analyses at Kamiura were sampled after each

Table 1 Foods used for rotifer enrichment and supplied to larval-rearing tanks without water exchange during the seed production of amberjack

	Food for rotifer enrichment		Food supplied to larval-rearing tank	
	Food	Enrichment conditions	Food	Supply/day
Kamiura	(S and L type) *Chlorella*[a]	20–28 h in 80% sea water at 23–26°C	*Chlorella*[b]	1
Kagoshima	(S type) *Chlorella*[a] + DHA emulsion[b]	17 h (*Chlorella*) + 4 h (DHA emulsion) in 80% sea water at 25°C	*Nannochloropsis*	2
	(L type) *Chlorella*[a]	17 h in 60% sea water at 25°C		
Miyazaki	(S type) *Nannochloropsis* and DHA emulsion[c]	17 h in 100% sea water at 24°C	*Nannochloropsis*[d]	1

[a] Super Fresh Chlorella V12 (SV12)

[b] Marine Glos

[c] Plus AQUARAN

[d] A commercial condensed product (Marine Fresh, Marine Bio, Kumamoto, Japan)

Table 2 Supply of enriched rotifers to larval-rearing tanks (+) and sampling of rotifers from the tanks (‡)[1] without water exchange during the seed production of amberjack[2]

	IFD (ind./t)[3]	WT (°C)[4]	Age (days after hatching) 2	3	4	5	6	7	8	9	10	11	12
Kamiura													
1st trial	5,000	26											
Enriched rotifer supply			+					+	+				
Tank rotifer sampling								‡			‡[5]		
2nd trial	5,000	26											
Enriched rotifer supply					+				+	+	+	+	
Tank rotifer sampling									‡				‡[5]
Kagoshima													
1st trial	4,900	26											
Enriched rotifer supply			+	+	+	+	+	+	+	+			
Tank rotifer sampling						‡					‡[5]		
2nd trial	8,200	26											
Enriched rotifer supply			+	+	+	+	+						
Tank rotifer sampling					‡			‡[5]					
Miyazaki													
1st trial	7,200	26											
Enriched rotifer supply			+	+	+	+	+	+					
Tank rotifer sampling							‡			‡			
2nd trial	8,300	26											
Enriched rotifer supply			+			+	+	+	+	+			
Tank rotifer sampling							‡			‡			

[1] Tank rotifers for chemical analyses were sampled before freshly enriched rotifers of the day were supplied to the tanks at all facilities, after each day's *Chlorella* was supplied to the tank at Kamiura, and before *Nannochloropsis* was supplied at Kagoshima and Miyazaki

[2] Duration without water supply in each trial is indicated as a *gray bar* (started at age 0)

[3] Initial fish density

[4] Water temperature in the larval-rearing tank

[5] Tank rotifers were sampled before the start of water supply

day's *Chlorella* supplementation and those at Kagoshima and Miyazaki before *Nannochloropsis* supplementation. The seed productions were conducted without duplication and resulted in relatively low survival rates (2–9%); we did not analyze the relationships between the growth and survival data and the nutrient contents of the rotifers.

Table 3 Lipid content and fatty acid profile of freshly enriched rotifers (*enriched*) and rotifers sampled from the larval tanks (*tank*)

	Lipids (% DM)			Fatty acids (area % in total lipids)	
	Total lipids	Neutral lipids	Polar lipids	20:5n-3	22:6n-3
Kamiura 1st trial					
Enriched ($n = 2$)	13.0 ± 1.1	6.9 ± 0.1	6.1 ± 1.2	3.4 ± 0.4	5.4 ± 1.0
Tank ($n = 2$)	11.6 ± 0.5	5.2 ± 0.2*	6.4 ± 0.4	5.2 ± 0.4	6.0 ± 1.3
2nd trial					
Enriched ($n = 3$)	12.0 ± 1.7	5.7 ± 1.3	6.4 ± 0.6	4.0 ± 0.7	5.7 ± 1.7
Tank ($n = 2$)	9.2 ± 3.2	4.1 ± 1.6	5.1 ± 1.6	4.5 ± 0.1	3.4 ± 1.4
Kagoshima 1st trial					
Enriched ($n = 2$)	12.4 ± 0.1	7.0 ± 0.7	5.4 ± 0.8	11.4 ± 0.3	8.3 ± 2.5
Tank ($n = 2$)	13.8 ± 1.8	8.2 ± 1.6	5.7 ± 0.1	12.7 ± 2.3	3.9 ± 1.6
2nd trial					
Enriched ($n = 2$)	16.0 ± 1.1	9.9 ± 0.1	6.2 ± 1.1	9.1 ± 5.8	10.3 ± 7.7
Tank ($n = 2$)	14.0 ± 1.8	8.4 ± 2.6	5.7 ± 0.8	13.0 ± 0.9	3.1 ± 0.8
Miyazaki 1st trial					
Enriched ($n = 3$)	15.4 ± 0.7	10.5 ± 0.5	5.4 ± 0.4	15.3 ± 2.1	3.9 ± 0.4
Tank ($n = 2$)	10.4 ± 2.6*	7.1 ± 2.5	3.2 ± 0.0*	19.9 ± 4.6*	0.8 ± 0.1*
2nd trial					
Enriched ($n = 3$)	17.1 ± 2.9	11.7 ± 2.4	5.3 ± 0.6	14.6 ± 2.9	5.6 ± 0.9
Tank ($n = 2$)	8.6 ± 2.0*	4.4 ± 0.6*	4.3 ± 1.3	15.8 ± 4.9	1.7 ± 1.1*

Values are mean ± SD

* Significant difference ($P < 0.05$) between the freshly enriched rotifers and the tank rotifers

Compared to the total lipid contents of the enriched rotifers, the contents of the tank rotifers tended to decrease except for the rotifers from the first trial at Kagoshima. However, significant reductions ($P < 0.05$) in the tank rotifers were observed only at Miyazaki (Table 3). The decrease seemed more pronounced in the neutral lipid contents than in the polar lipid contents. The relative proportions of 20:5n-3 in the total lipids tended to increase whereas those of 22:6n-3 decreased, although a significant difference was observed only for 20:5n-3 in the first trial and 22:6n-3 in both trials at Miyazaki, due to the large variability among the samples.

The profiles of selected fatty acids in the neutral lipids are shown in Fig. 1. The proportions of total saturated fatty acids (saturates) were not significantly different between the enriched rotifers and the tank rotifers. The proportions of total mono-unsaturated fatty acids (monoenes) decreased significantly in the tank rotifers for both trials at Miyazaki, while those of 18:2n-6 decreased in the tank rotifers at Kamiura. The proportions of 20:5n-3 tended to increase in the tank rotifers relative to the enriched ones, but the difference was significant only in the first trial at Kamiura. Although the proportions of 22:6n-3 between the rotifers at Kamiura were not significantly different, the proportions in the tank rotifers taken at the other facilities, especially those at Miyazaki, were significantly lower compared to the enriched ones.

The proportions of selected fatty acids in the polar lipids are presented in Fig. 2. The overall trends in the changes of fatty acid profiles between the enriched and tank rotifers

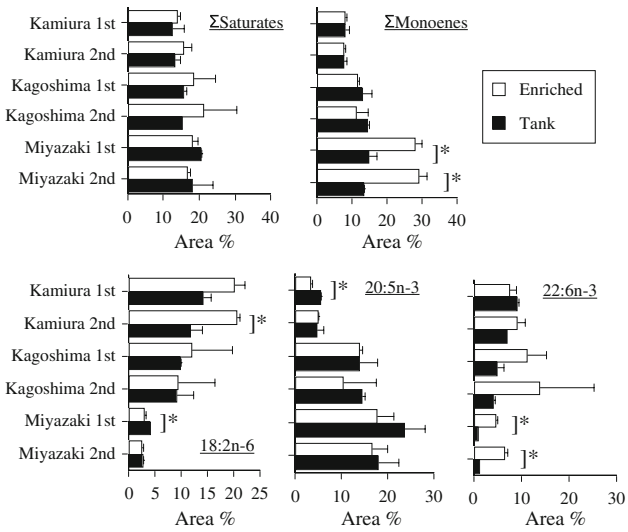

Fig. 1 Relative proportions of selected fatty acids (area %) in the neutral lipids of the freshly enriched rotifers (*white*) and the tank rotifers sampled from the larval-rearing tanks (*black*). Values are mean ± SD. The sample sizes are shown in Table 3. An *asterisk* means a significant difference ($P < 0.05$) between the enriched rotifers and the tank rotifers

were similar to those observed in the neutral lipids. However, the extent of the decrease for total monoenes (at Miyazaki) and 18:2n-6 (at Kamiura) in the tank rotifers seemed milder relative to the cases in the neutral lipids. Moreover, the proportion of 22:6n-3 in the tank rotifers in the first trial at Kamiura was significantly higher than the enriched ones.

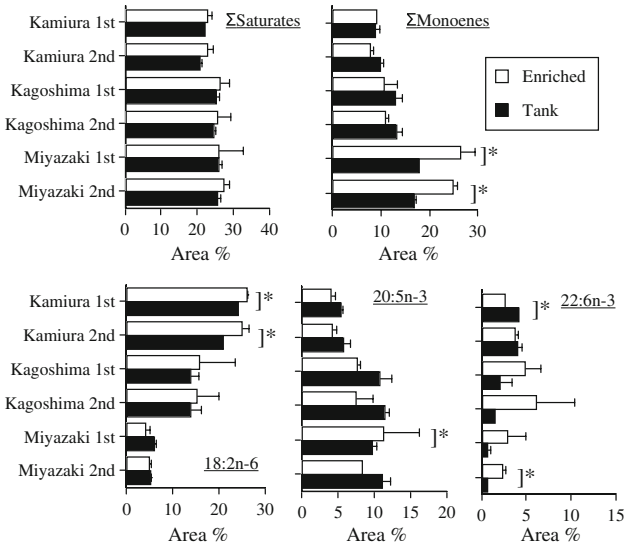

Fig. 2 Relative proportions of selected fatty acids (area %) in the polar lipids of the freshly enriched rotifers (*white*) and the tank rotifers (*black*). Values are mean ± SD. The sample sizes are shown in Table 3. An *asterisk* means a significant difference ($P < 0.05$) between the enriched rotifers and the tank rotifers

The crude protein contents were not significantly different between the enriched rotifers and the tank rotifers although the contents of the tank rotifers at Miyazaki tended to decrease (Table 4). The sum of total amino acids determined by the hydrochloric acid digestion almost paralleled the crude protein content. The relative proportions of the sum of essential amino acids (percent essential amino acids/all amino acids) slightly decreased in the tank rotifers, a result that was significant in the first trial at Kagoshima and both trials at Miyazaki. Among the essential amino acids, the proportions of lysine tended to decrease in the tank rotifers whereas those of proline increased (Fig. 3). In the aqueous extracts, the contents of taurine were not significantly different between the enriched and tank rotifers (Table 4). The sum of the content of free amino acids in the tank rotifers at Miyazaki increased relative to the levels of the enriched ones unlike the trends observed at the other facilities. There were no specific changes in the content of individual free amino acids between the enriched and tank rotifers (results not shown).

Significant changes in the vitamin C and E contents between the enriched and tank rotifers were observed only at Miyazaki (Table 5). The vitamin C content in the tank rotifers decreased markedly from the levels of the enriched ones (only 13 and 30% of the enriched rotifers in the respective trials). The vitamin E content of the tank rotifers in the second trial at Miyazaki also decreased to 50% of the enriched rotifers.

Compared to the levels of the enriched rotifers, contents of minerals examined generally increased in the tank rotifers except for phosphorus and magnesium (Table 6). Among them, the increases in calcium at Kamiura; calcium, iron, and manganese at Miyazaki; and zinc at Kagoshima were pronounced in the tank rotifers. By contrast, the phosphorus contents in the tank rotifers tended to decrease slightly relative to the contents of the enriched rotifers.

Table 4 Protein content and amino acid profiles of freshly enriched rotifers (*enriched*) and rotifers sampled from the larval tanks (*tank*) (dry-matter basis)

	Crude protein (g/100 g)	Total amino acids		Free amino acids (mg/g)	
		∑AA[a] (g/100 g)	%∑EAA[a]	Taurine	∑AA[a]
Kamiura 1st trial					
Enriched ($n = 2$)	65.9 ± 4.0	49.7 ± 3.2	49.8 ± 0.3	1.8 ± 0.4	11.3 ± 0.9
Tank ($n = 2$)	66.9 ± 1.8	51.6 ± 1.5	49.4 ± 0.3	1.7 ± 0.1	13.1 ± 0.6
2nd trial					
Enriched ($n = 3$)	66.4 ± 1.6	50.1 ± 0.9	49.5 ± 0.1	1.3 ± 0.2	13.1 ± 4.4
Tank ($n = 1, 2$)[b]	63.1	48.3	48.9	1.6 ± 0.4	7.3 ± 0.1
Kagoshima 1st trial					
Enriched ($n = 2$)	65.5 ± 1.5	51.6 ± 0.9	50.3 ± 0.3	1.3 ± 0.2	17.8 ± 3.3
Tank ($n = 2$)	66.8 ± 2.1	54.3 ± 0.1	49.3 ± 0.2*	1.4 ± 0.5	21.0 ± 2.8
2nd trial					
Enriched ($n = 2$)	63.5 ± 5.0	51.3 ± 4.5	50.1 ± 0.2	1.2 ± 0.2	24.0 ± 10.1
Tank ($n = 2$)	64.4 ± 1.0	50.7 ± 1.3	49.2 ± 0.5	1.3 ± 0.0	22.6 ± 8.3
Miyazaki 1st trial					
Enriched ($n = 3$)	67.6 ± 1.8	53.6 ± 1.1	50.2 ± 0.2	1.5 ± 0.0	22.3 ± 1.8
Tank ($n = 2$)	63.7 ± 3.0	52.1 ± 2.2	49.7 ± 0.0*	1.5 ± 0.2	45.6 ± 12.3*
2nd trial					
Enriched ($n = 3$)	65.6 ± 1.1	53.1 ± 1.3	50.4 ± 0.2	1.3 ± 0.0	17.9 ± 0.3
Tank ($n = 2$)	56.6 ± 9.0	44.3 ± 7.9	49.5 ± 0.4*	1.4 ± 0.1	25.2 ± 8.5

Values are mean ± SD

[a] ∑AA indicates the sum of all amino acids, %∑EAA = 100 × sum of essential amino acids/sum of all amino acids

[b] One sample was analyzed for crude protein content and total amino acid profile

* Significant difference ($P < 0.05$) between the freshly enriched rotifers and the tank rotifers

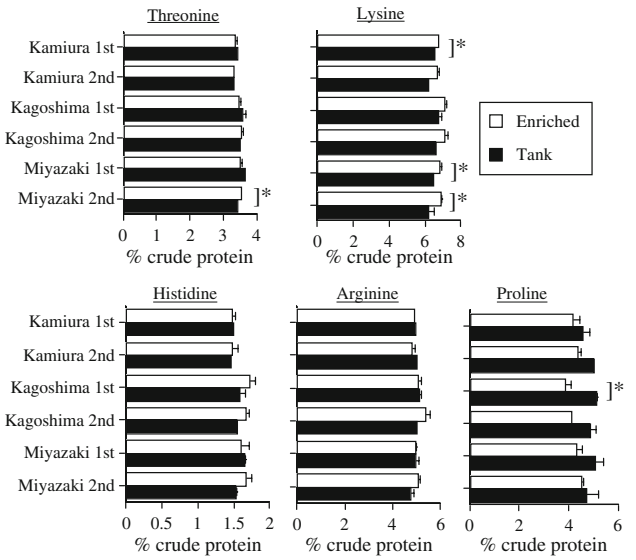

Fig. 3 Relative proportions of selected amino acids (% crude protein) determined by acid hydrolysis of the freshly enriched rotifers (*white*) and the tank rotifers (*black*). Values are mean ± SD. The sample sizes are shown in Table 4. An *asterisk* means a significant difference ($P < 0.05$) between the enriched rotifers and the tank rotifers

Discussion

In laboratory experiments simulating a stagnant larval-rearing tank where rotifers were kept without fish larvae, the addition of microalgae was found to almost maintain the contents of lipids and essential fatty acids (20:5n-3 or 22:6n-3 depending on the fatty acid profile of the supplemented microalga) in the rotifers [1, 2]. In the present study, lipid contents in the tank rotifers sampled from the amberjack larval-rearing tanks were similar (reduced at most by 23%) to the respective enriched rotifers at Kamiura and Kagoshima, while the contents of the tank rotifers at Miyazaki markedly decreased by 32–50%. Moreover, the relative proportions of 22:6n-3 in the total lipids of the tank rotifers at Kamiura were also similar to the enriched rotifers. However, the proportions of 22:6n-3 in the tank rotifers at Kagoshima and Miyazaki tended to decrease with the increasing trends in the proportions of 20:5n-3. It is well established that the fatty acid composition of rotifers reflects the composition of their food [7, 14–17]. In addition, in wild zooplankton, vitamin C has been found to be derived from phytoplankton [18], and microalgae such as *Chlorella* and *Nannochloropsis* are good sources of vitamin C for rotifers [8, 9]. The vitamin C contents of the tank rotifers at Miyazaki decreased markedly compared to the enriched ones although the contents of the tank rotifers at Kamiura and Kagoshima did not decrease significantly.

Considering that (1) the microalgae supplemented to the larval-rearing tanks were *Chlorella* SV12 (22:6n-3 rich) at Kamiura and *Nannochloropsis* (20:5n-3 rich) at Kagoshima and Miyazaki, (2) the tank rotifers were sampled for the chemical analyses after the SV12 supplementation of the day to the larval-rearing tank at Kamiura and before the *Nannochloropsis* supplementation at Kagoshima and Miyazaki, and (3) *Nannochloropsis* was supplemented twice daily to hold its concentration in the larval-rearing tank as constant as possible at Kagoshima (Table 1 and 2), the differences observed in the lipid contents, fatty acid profiles, and vitamin C contents of the tank rotifers among the three facilities could be attributable to the kind of microalgae supplemented to the larval tank and the microalgae concentration in the larval tank at the sampling of the rotifers. In other words, the tank rotifers sampled for the chemical analyses at Kamiura and Kagoshima might have been less starved than at Miyazaki, which resulted in the lipid and vitamin C contents of the tank rotifers at Kamiura and Kagoshima being maintained at higher levels than at Miyazaki. This further leads to the conclusion, together with the findings under the laboratory conditions without fish larvae mentioned above [1, 2], that the lipid and vitamin C status of the tank rotifers could be maintained, not always fully but to a large extent, by appropriate supplementation of microalgae (microalga species and maintaining the concentration in the larval-rearing tank).

Table 5 Vitamin C and E contents of freshly enriched rotifers (*enriched*) and rotifers sampled from the larval tanks (*tank*) (dry-matter basis)

	Vitamin C (mg/100 g)	Vitamin E (mg/100 g)
Kamiura 1st trial		
Enriched ($n = 2$)	46 ± 7	11 ± 3
Tank ($n = 2$)	72 ± 4	12 ± 5
2nd trial		
Enriched ($n = 3$)	48 ± 3	18 ± 9
Tank ($n = 1$)	51	15
Kagoshima 1st trial		
Enriched ($n = 2$)	152 ± 48	29 ± 7
Tank ($n = 2$)	95 ± 6	27 ± 1
2nd trial		
Enriched ($n = 2$)	119 ± 80	18 ± 10
Tank ($n = 2$)	135 ± 44	38 ± 1
Miyazaki 1st trial		
Enriched ($n = 3$)	108 ± 14	23 ± 3
Tank ($n = 2$)	14 ± 7*	17 ± 1
2nd trial		
Enriched ($n = 3$)	110 ± 2	28 ± 0
Tank ($n = 2$)	33 ± 17*	14 ± 4*

Values are mean ± SD

* Significant difference ($P < 0.05$) between the freshly enriched rotifers and the tank rotifers

Table 6 Contents of selected minerals of freshly enriched rotifers (*enriched*) and rotifers sampled from the larval tanks (*tank*) (dry-matter basis)

	Ca (mg/g)	P (mg/g)	Mg (mg/g)	Fe (μg/g)	Mn (μg/g)	Zn (μg/g)
Kamiura 1st trial						
Enriched ($n = 2$)	1.4 ± 0.4	10.7 ± 0.7	2.8 ± 0.2	144 ± 26	7.1 ± 0.6	51 ± 14
Tank ($n = 2$)	6.0 ± 0.4*	9.6 ± 0.4	2.7 ± 0.0	262 ± 34	11.7 ± 1.8	59 ± 10
Kagoshima 1st trial						
Enriched ($n = 2$)	1.5 ± 0.0	12.6 ± 0.3	3.9 ± 0.7	129 ± 8	12.1 ± 6.4	53 ± 5
Tank ($n = 2$)	2.2 ± 0.4	11.1 ± 0.3*	2.3 ± 0.3	222 ± 27*	11.9 ± 0.2	317 ± 84*
Kagoshima 2nd trial						
Enriched ($n = 2$)	1.3 ± 0.6	11.6 ± 1.4	3.3 ± 1.2	111 ± 15	8.3 ± 0.2	48 ± 6
Tank ($n = 2$)	2.6 ± 1.1	10.8 ± 0.1	2.4 ± 0.5	195 ± 19*	14.5 ± 4.6	498 ± 113*
Miyazaki 1st trial						
Enriched ($n = 3$)	1.0 ± 0.1	12.0 ± 0.9	2.1 ± 0.3	93 ± 19	7.3 ± 0.6	55 ± 7
Tank ($n = 2$)	21.3 ± 8.5*	11.7 ± 0.3	3.3 ± 0.2*	2383 ± 626*	23.4 ± 1.2*	77 ± 5*

Values are mean ± SD

* Significant difference ($P < 0.05$) between the freshly enriched rotifers and the tank rotifers

The decrease in the lipid contents of the tank rotifers is due to starvation as has been previously reported [1, 2, 7]. In this study, the decrease was more pronounced in the neutral lipids relative to the polar lipids, suggesting that lipid reserves (neutral lipids) of the tank rotifers were preferentially used for energy production. Similar trends were also observed in the changes in fatty acid profiles between the neutral and polar lipids, although caution should be paid to the changes in the lipid contents of the rotifers, fatty acid profile of the supplemental microalgae, and the extent of starved conditions (food restriction), since the relative proportions of essential fatty acids (20:5n-3 and 22:6n-3) in the rotifers under complete starvation did not change markedly [1, 7].

The decreases in the proportions of 22:6n-3 in the tank rotifers at Kagoshima and Miyazaki may be due to the supplemental *Nannochloropsis* (lacking in 22:6n-3) added to the larval-rearing tanks. In addition, monoenes are rich in *Nannochloropsis* [17] while 18:2n-6 is rich in *Chlorella* [17, 19]. In the tank rotifers, marked decreases in the proportion of 18:2n-6 were observed at Kamiura and of monoenes at Miyazaki, with the same kind of microalgae being used both for enrichment and tank supplementation, respectively (*Chlorella* SV12 and *Nannochloropsis*). Therefore, these fatty acids are considered to be principally used for energy production under the food-restricted conditions in the larval-rearing tanks.

In contrast to the changes in the lipid content and fatty acid profile of the tank rotifers, the changes in the crude protein contents and the free and total amino acid profiles were less pronounced in the present study. No significant changes were observed in the crude protein content between the enriched and tank rotifers, although decreasing trends were observed in the tank rotifers at Miyazaki, which are considered to have been sampled at the most severely food-restricted phases among the three facilities. In the tank rotifers at Miyazaki, the contents of free amino acids increased and the proportion of lysine in the total amino acids decreased, unlike the cases of starved rotifers in which the relative proportion of protein-bound essential amino acids increased and the content of free amino acids decreased [4].

Under starved conditions, changes in the protein-bound and free amino acid profiles are likely to occur by selective catabolism of some body proteins of rotifers for maintenance and energy production [4]. In *Calanus finmarchicus*, the decrease in protein content during starvation was found to be moderate during the first 10 days but then drastically worsen during the following 21 days [20], suggesting that proteins are saved at the expense of lipids under moderately-starved conditions. In starved rotifers, the relative protein content (percent dry matter) increased [4], however, this is probably merely an apparent increase due to the decrease in lipid content. In another study, the protein content in individual rotifers decreased during starvation, but the protein content was maintained by addition of *Tetraselmis* sp. [3]. In addition, protein-bound amino acid profiles of rotifers have been shown to be little affected by their foods [4, 14]. Thus, we conclude that protein status in tank rotifers is also not critical when microalgae, regardless of the species, are appropriately added to larval-rearing tanks.

The contents of minerals in the enriched rotifers were similar across the three facilities. This is in agreement with a previous finding that mineral contents of live food organisms artificially cultivated are only minimally

affected by their foods [14]. By contrast, except for phosphorus and magnesium, the contents of other minerals examined generally increased in the tank rotifers, especially the calcium content at Kamiura and Miyazaki, the iron and manganese contents at Miyazaki, and the zinc content at Kagoshima. In contrast to the cases observed in lipids, these phenomena cannot be explained by the extent of the food restriction to the rotifers or the kind of microalgae supplemented. Although we did not analyze the mineral compositions of either the fresh sea water initially supplied to the tanks or the larval tank water, mineral compositions of live food organisms are suggested to be affected by the mineral contents of rearing waters [14]. On the other hand, certain microminerals such as manganese and zinc in rotifers have been suggested to be deficient for marine fish larvae compared to the requirements of larger fish as well as the contents in wild-caught copepods [21].

Skeletal disorders that are often found in hatchery-reared fish have been a serious problem, and insufficiency or excess of microminerals as well as an imbalance in the calcium-to-phosphorus ratio in food organisms and feed have been suggested as potential causes of the occurrence of skeletal malformations [22]. In the seed production of amberjack, the occurrence of skeletal deformities is also a serious problem to be solved. Further precise investigations on the relationship between the mineral contents of live foods including tank rotifers and the skeletal development of amberjack larvae are necessary.

Conclusion

In conclusion, the lipid, protein, and vitamin (C and E) status of tank rotifers that survived in larval-rearing tanks without water supply during amberjack seed production could be maintained to a large extent by appropriate supplementation (species and concentration) of microalgae to the tank. The effect of the accumulation of certain minerals in the tank rotifers on fish growth and skeletal development as well as the origin of the minerals should be investigated in more detail.

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