藍藻Synechocystis sp.SY-4で栄養強化したシオミズツボワムシおよびアルテミアのマダイ仔魚,オニオコゼ仔魚およびヒラメ仔魚に対する餌料価値

誌名	水産増殖 = The aquiculture
ISSN	03714217
著者	阪本, 憲司 沖増, 英治 雨村, 明倫
巻/号	45巻3号
掲載ページ	p. 365-370
発行年月	1997年9月

(水産増殖 45巻3号 365−370)
(SUISANZOSHOKU (1997-H 9))

Dietary Value of the Rotifer and the Brine Shrimp Enriched with *Synechocystis* sp. SY-4 as Feed for Larvae of Red Sea Bream, Devil Stinger, and Japanese Flounder

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Abstract

The dietary value of the rotifer, *Brachionus rotundiformis*, and the brine shrimp, *Artemia salina*, enriched with a blue-green alga, *Synechocystis* sp. SY-4, was evaluated by feeding them to the larvae of red sea bream, *Pagrus major*, devil stinger, *Inimicus japonicus*, and Japanese flounder, *Paralichthys olivaceus*. Results were compared to those of zooplankton enriched with *Nannochloropsis oculata*, *Euglena gracilis* and *Chlorella vulgaris* K-22, singly or in combination with *Synechocystis* sp. SY-4.

The survival rate and growth of devil stinger fed on zooplankton enriched with *Synechocystis* sp. SY-4 was better than those on other diet. The dietary value of rotifer enriched only with *Synechocystis* sp. SY-4 for the larvae of red sea bream and Japanese flounder was low, but was effectively improved by combination with *N. oculata* or *E. gracilis*.

A blue-green alga (cyanobacterium), *Synechocystis* sp. SY-4, was isolated from the Mekari Strait, Seto Inland Sea.[1] It can grow at temperature between 25 and 35 ℃ in a simple economical medium for mass culture consisting of three fertilizers (ammonium sulfate, calcium perphosphate and Clewat-32), and was found to be a useful feed for the culture of rotifer used for biomass production, giving better growth of rotifer than other microalgae (*Tetraselmis tetrathele* and *Nannochloropsis oculata*) generally used as feed for zooplankton.[1] However, whether or not rotifers cultured with this microalga will satisfy the dietary needs of fish larvae remained to be determined. In this study, their dietary value was evaluated by feeding them to the larvae of red sea bream, devil stinger and Japanese flounder.

Materials and Methods

Fish and rearing conditions

Red sea bream

Red sea bream eggs, naturally fertilized on May 8, 1995, were obtained from the Hiroshima Prefectural Mariculture Center, Takehara, Hiroshima, and incubated at 20 ℃ in a 200 *l* polycarbonate tank at the Research Institute of Marine Bioresources, Fukuyama University, on May 9. Then, the eggs were divided into six groups, each comprising 6,000 eggs, and kept in six 200 *l* experimental tanks (Tanks A_1-C_2). On May 11, the eggs hatched (about 98% hatching rate) and the developed larvae were reared with rotifers for 25

Received : March 21, 1997
Key words : *Synechocystis* sp. SY-4, dietary value

days as described below. The seawater in the experimental tanks was aerated with air stones at a rate of 200 ml/min and exchanged continuously at a rate of 1 turnover/day. The mean water temperature during the experiment was 21.1℃ (19.7-22.1℃).

Rotifers, which had been cultured with *Tetraselmis tetrathele*, were enriched (2-8 h) with a sufficient amount of *Synechocystis* sp. SY-4 (S), *Synechocystis* sp. SY-4 and *Nannochloropsis oculata* (SN), or *N. oculata* (N), and then introduced into the experimental tanks. The feeding density of rotifers given to the larvae was maintained at 10 ind./ml in the tanks by feeding them five times a day.

Larval growth was determined by measuring the total lengths of 30 larvae 15 and 25 days after hatching. The survival rate was calculated every day by counting dead larvae.

Devil stinger

Devil stinger eggs, spawned from wild fish and fertilized on June 24, 1995, at the Research Institute of Marine Bioresources, Fukuyama University, were incubated in a 500 *l* polycarbonate tank at 28℃. Then, the eggs were divided into ten groups of 3,000 each and kept in ten 200 *l* experimental tanks (Tanks A_1-E_2). On June 25, the eggs hatched (about 98.5% hatching rate). The larvae were reared with rotifers and brine shrimps for 18 days as described below. The seawater in the experimental tanks was aerated with air stones at a rate of 200 ml/min and exchanged continuously at a rate of 1 turnover/day. The mean water temperature was 28.4℃ (27.6-29.0℃).

Rotifers which had been cultured with *Chlorella vulgaris* K-22 (*Chlorella*-V_{12}, Chlorella Ind. Co., Ltd.) and brine shrimps (America strain) were enriched (2-8 h) with a sufficient amount of *Synechocystis* sp. SY-4 (S), *Synechocystis* sp. SY-4 and *Euglena gracilis* (Docosa-*Euglena*, Harima Chemicals Inc.) (SE), *E. gracilis* (E), *E. gracilis* and *Chlorella vulgaris* K-22 (*Chlorella*-ω3, Chlorella Ind. Co., Ltd.) (EC), or *Chlorella*-ω3 (C), and then introduced into the experimental tanks. The rotifers were fed at a density of 10 ind./ml alone and 5 ind./ml in combination with brine shrimps (0.5 ind./ml) from day 1 to day 4, and from day 5 to day 9, respectively. From day 10, onwards the brine shrimps were fed alone at a density of 1 ind./ml. Their density in the tank was maintained by feeding them five times a day.

Thirty larvae were sampled from each tank at day 10 and day 18, and their total lengths were determined. The survival rate was calculated every day by counting dead larvae.

Japanese flounder

Japanese flounder eggs, naturally fertilized on October 10, 1995, were obtained from Marua Suisan Co., Ltd., Iwagi, Ehime. The eggs were incubated in a 500 *l* polycarbonate tank at 18℃. Then, the eggs were divided into 10 groups of 7,500 each and distributed into 500 *l* experimental tanks (Tanks A_1-E_2). On October 12, the eggs hatched (about 98% hatching rate). The larvae were reared with rotifers for 30 days.

Rotifers which had been cultured with *T. tetrathele* were enriched (2-8 h) with a sufficient amount of *Synechocystis* sp. SY-4 (S), *Synechocystis* sp. SY-4 and *E. gracilis* (SE), *Synechocystis* sp. SY-4 and *N. oculata* (SN), *E. gracilis* (E), or *N. oculata* (N), and then introduced into the experimental tanks. The feeding density of rotifers was maintained at 10 ind./ml.

The rearing water was aerated and exchanged under the same conditions described above. The mean water temperature during the experiment was 18.8℃ (17.0-22.0℃).

Thirty larvae were sampled to determine the total length at day 20 and 30. The survival rate was calculated every day.

Statistical analyses of the data were conducted by means of Student's *t*-test.

Results and Discussion

Red sea bream

The growth and survival of the larval red sea bream fed on rotifers enriched with algae are shown in Table 1. Growth of larvae fed on SN-enriched rotifers was the highest and those fed on S-enriched rotifers was the lowest at day 25 (SN = N > S; $p < 0.05$). The survival rate of larvae fed on N-enriched rotifers was slightly higher than that of the others at 25 days. Thus, the dietary value of S-enriched rotifers was rather inferior compared to that of N-enriched

Table 1. Growth and survival rate of the larval red sea bream fed on rotifers that had been enriched with *Synechocystis* sp. SY-4 (S), a combination of *Synechocystis* sp. SY-4 and *N. oculata* (SN), and *N. oculata* (N) *1

Larval age (days)	Rotifers enriched with	Tank	Total length (mm)		Survival rate (%)
			Mean	(SD)	
15	S	A_1	4.7	(0.3)	96.1
		A_2	4.8	(0.3)	96.5
	SN	B_1	5.3*2	(0.4)	97.7
		B_2	5.3*2	(0.4)	97.5
	N	C_1	5.1	(0.4)	98.8
		C_2	5.2	(0.3)	98.3
25	S	A_1	6.8	(0.7)	93.8
		A_2	6.8	(0.6)	94.2
	SN	B_1	7.6*2	(0.7)	94.5
		B_2	7.5*2	(0.6)	94.8
	N	C_1	7.3	(0.6)	97.0
		C_2	7.4	(0.5)	96.7

*1 Data were based on 30 specimens sampled at random from each group.
*2 Not significant, compared with the values of N ($p<0.05$).

rotifers. However, the dietary value of S-enriched rotifers was increased by means of a mixed culture with *N. oculata*.

Marine finfish larvae were shown to require 0.3-0.5% of n-3 highly unsaturated fatty acids (HUFAs) in food organisms,[2] and larvae of red sea bream to require more than 0.3% of n-3 HUFA in a rotifer feed.[3] The level of n-3 HUFA in rotifers cultured with *N. oculata* was sufficient for the rearing of marine finfish larvae.[4-9] However, the level of n-3 HUFA in rotifers cultured with *Synechocystis* sp. SY-4 was not sufficient (0.16%) as shown in Table 2. Therefore, the nutritive value of the rotifers must have been improved by combination with *N. oculata*.

Devil stinger

The growth and survival of the larval devil stinger fed on rotifers and brine shrimps enriched with algae is shown in Table 3. The growth of larvae fed on S-enriched feed was the highest at day 18 (S > SE = E = C = EC; $p < 0.005$), and the survival rate of this group was slightly higher than those of the other groups at day 18. Thus, the dietary value of the S-enriched feed for larval devil stinger was high.

Larvae of devil stinger were reported to require the enrichment of HUFA in rotifers and brine shrimps.[11,12] The *E. gracilis* and *Chlorella*-ω3 used in this experiment had been enriched with n-3 HUFA to more than 73% and 28% of total fatty acids,

Table 2. Fatty acid composition of the rotifer cultured with *Synechocystis* sp. SY-4 *1

Fatty acid	(Area %) *3
12 : 0	——*4
14 : 0	0.71
15 : 0	0.71
16 : 0	14.62
16 : 1 n-7	0.93
16 : 1 n-5	2.23
16 : 2	1.26
16 : 3 n-6	——
16 : 3 n-3	——
18 : 0	4.49
18 : 1	36.52
18 : 2 n-6	9.48
18 : 3 n-6	——
18 : 3 n-3	2.10
18 : 4 n-3	0.68
20 : 0	1.76
20 : 1	4.06
20 : 2	4.96
20 : 4	0.82
20 : 5 n-3	3.99
22 : 0	0.43
22 : 1	0.33
22 : 4 n-9	0.13
22 : 4 n-6	1.07
22 : 5 n-3	0.88
22 : 6 n-3	0.40
24 : 1	0.24
Σn-3 HUFA *2	0.16
Total fatty acid *2	1.89

*1 Sakamoto, K. *et al.* (1995)[10]
*2 Expressed as a percentage of the dry weight of rotifer.
*3 Expressed as a percentage of the total fatty acid.
*4 Not detected.

Table 3. Growth and survival rate of the larval devil stinger fed on rotifers and brine shrimps enriched with *Synechocystis* sp. SY-4 (S), a combination of *Synechocystis* sp. SY-4 and *E. gracilis* (SE), *E. gracilis* (E), a combination of *E. gracilis* and *C. vulgaris* (EC), and *C. vulgaris* (C) *1

Larval age (days)	Rotifers and brine shrimps enriched with	Tank	Total length (mm)		Survival rate (%)
			Mean	(SD)	
10	S	A_1	6.6	(0.3)	91.5
		A_2	6.5	(0.4)	91.7
	SE	B_1	6.3	(0.3)	89.9
		B_2	6.3	(0.3)	90.7
	E	C_1	6.1	(0.3)	87.3
		C_2	6.0	(0.3)	87.2
	EC	D_1	5.8	(0.3)	90.0
		D_2	5.9	(0.4)	89.7
	C	E_1	5.9	(0.4)	85.4
		E_2	6.0	(0.3)	85.3
18	S	A_1	11.3*2	(0.8)	86.4
		A_2	11.3*2	(0.8)	86.7
	SE	B_1	10.7	(0.9)	86.3
		B_2	10.6	(0.8)	86.1
	E	C_1	10.3	(0.9)	83.7
		C_2	10.3	(0.8)	83.1
	EC	D_1	10.0	(0.9)	86.0
		D_2	10.1	(0.8)	85.8
	C	E_1	10.0	(0.8)	78.2
		E_2	10.2	(0.9)	77.0

*1 Data were based on 30 specimens sampled at random from each group.
*2 Significant, compared with the values of SE, E, EC and C ($p < 0.005$).

respectively, indicating that the diets enriched with these algae satisfy the HUFA requirement of devil stinger larvae. On the other hand, the level of n-3 HUFA in rotifers cultured with *Synechocystis* sp. SY-4 was not as high as described previously. The well growth and higher survival rate obtained with SY-4 in this experiment may indicate that the requirement of n-3 HUFA for devil stinger larvae is lower than those of other marine finfish larvae.

Rotifers cultured with *Synechocystis* sp. SY-4 contained a high level of oleic acid (36.5% of total fatty acid) (Table 2), and the protein content of *Synechocystis* sp. SY-4 was also high, about 48% on a dry basis.[10] The high contents of these nutrients in the diet may influence the growth of the larval devil stinger.

Japanese flounder

The results of growth and survival of the larval Japanese flounder are shown in Table 4. The growth of the larvae fed on SE-enriched rotifers was the highest and those fed on S-enriched rotifers was the lowest at day 30 (SE > SN = N = E > S; $p < 0.002$), but the survival rate of the SE-enriched rotifer group was slightly lower than those of the SN-, E- and N-enriched rotifer groups at day 30. The survival of the S-enriched rotifer group was the lowest. These results show that the dietary value for the S-enriched rotifer group was rather lower than to those for the other groups, but was increased by means of a mixed culture with *E. gracilis* or *N. oculata*.

The growth of larvae of Japanese flounder also require n-3 HUFA as essential fatty acids, among them more docosahexaenoic acid (DHA; 22:6 n-3) (1%) than eicosapentaenoic acid (EPA; 20:5 n-3) required.[13] However, the level of DHA in rotifers cultured with *Synechocystis* sp. SY-4 was not sufficient (0.4% of total amount of fatty acids) (Table 2). This may explain that the dietary value for rotifers enriched with *Synechocystis* sp. SY-4 only was low for the larvae of Japanese flounder. Thus, the value for the rotifers must be improved by combination culture with

Table 4. Growth and survival rate of the larval Japanese flounder fed on rotifers enriched with *Synechocystis* sp. SY-4 (S), a combination of *Synechocystis* sp. SY-4 and *E. gracilis* (SE) or *N. oculata* (SN), *E. gracilis* (E), and *N. oculata* (N) *1

Larval age (days)	Rotifers enriched with	Tank	Total length (mm)		Survival rate (%)
			Mean	(SD)	
20	S	A_1	7.9	(0.4)	94.0
		A_2	7.9	(0.3)	94.3
	SE	B_1	9.4*2	(0.4)	97.9
		B_2	9.3*2	(0.4)	97.6
	SN	C_1	8.8	(0.4)	97.0
		C_2	8.7	(0.4)	96.8
	E	D_1	8.3	(0.3)	95.8
		D_2	8.2	(0.4)	96.0
	N	E_1	8.2	(0.4)	98.5
		E_2	8.3	(0.3)	98.3
30	S	A_1	9.8	(0.9)	88.8
		A_2	9.8	(0.9)	88.4
	SE	B_1	12.5*2	(0.9)	91.8
		B_2	12.5*2	(1.0)	92.0
	SN	C_1	11.6	(0.9)	94.2
		C_2	11.7	(0.9)	94.4
	E	D_1	10.7	(0.8)	94.7
		D_2	10.6	(0.8)	94.9
	N	E_1	11.1	(0.9)	94.5
		E_2	11.2	(0.8)	94.7

*1 Data were based on 30 specimens sampled at random from each group.
*2 Significant, compared with the values of S, SN, E and N ($p<0.002$).

E. gracilis, which contains a high level of DHA, as shown here.

In this experiment, the dietary value of the rotifer and the brine shrimp, enriched with a blue-green alga, *Synechocystis* sp. SY-4, was evaluated by feeding them to the larvae of red sea bream, devil stinger and Japanese flounder. Our results showed that the survival rate and growth of the larval devil stinger fed on the zooplanktons enriched with *Synechocystis* sp. SY-4 was good, the dietary value for red sea bream and Japanese flounder fed on the rotifer enriched with *Synechocystis* sp. SY-4 was low, but could be effectively improved by combination with *N. oculata* or *E. gracilis*.

Acknowledgement

We wish to thank the Hiroshima Prefectural Mariculture Center, Takehara, Hiroshima, and Marua Suisan Co., Ltd., Iwagi, Ehime for providing us with the eggs of the red sea bream and Japanese flounder, respectively.

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藍藻 *Synechocystis* sp. SY-4で栄養強化したシオミズツボワムシおよびアルテミアのマダイ仔魚，オニオコゼ仔魚およびヒラメ仔魚に対する餌料価値

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藍藻 *Synechocystis* sp. SY-4で栄養強化したシオミズツボワムシ（以下ワムシと略す）およびアルテミアのマダイ仔魚，オニオコゼ仔魚およびヒラメ仔魚に対する餌料価値を調べた。*Synechocystis* sp. SY-4の餌料価値を評価するために，*Nannochloropsis oculata*, *Euglena gracilis*（ドコサ・ユーグレナ，ハリマ化成㈱）および *Chlorella vulgaris* K-22（*Chlorella*-ω3，クロレラ工業㈱）を対照餌料として用いた。その結果，*Synechocystis* sp. SY-4で栄養強化したワムシおよびアルテミアのオニオコゼ仔魚に対する餌料価値は，高く評価された。また，マダイ仔魚およびヒラメ仔魚に対しては，*N. oculata*や*E. gracilis*と併用することで，その餌料価値を更に高める効果が得られた。