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Effects of rotifer, *Brachionus rotundiformis* in enhancing some digestive enzyme activities into glucose on somatic growth, RNA/DNA contents and some digestive enzyme activities of olive flounder *Paralichthys olivaceus* larvae

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We investigated the effects of enzyme enhanced rotifer (EER), *Brachionus rotundiformis* on growth, RNA/DNA contents and digestive enzyme activities in olive flounder *Paralichthys olivaceus* larvae. Digestive enzyme activities of rotifer were enhanced by putting glucose in culture medium, and then rotifers (NR, rotifer not supplied the glucose during culture, and EER) was supplied to 10 rotifers/ml in larval rearing tank twice daily. The length-daily growth rate (DGR) showed a sudden decrease at 7 days post hatch (dph), and the weight DGR showed two times decreasing at 5 and 8 dph. The larvae in the NR treatment underwent two times the decrease of daily growth rate of body weight, while those in the EER treatment underwent only the first decrease of that. The DNA content for both treatment increased constantly. The RNA content increased in the larvae measuring up to 3.7 mm in the NR treatment. However, the RNA content of the EER treatment increased in the larvae until the end of the experiment. Therefore, supplement of rotifer enhanced the digestive enzyme activities for early olive flounder larvae prevented the decrease of RNA contents related with a protein synthesis and body weight growth after 7 dph, because NR is not enough for early larvae.

Key words: Enzyme enhanced activity, nucleic acid content, somatic growth, critical period, flounder larvae, *Paralichthys olivaceus*, rotifer.

INTRODUCTION

The research on growth patterns as a physiological duality in fish larvae has mainly focused on resource accumulation (Nakano and Shirahata, 1988). These researches investigated the effect of food and species in coastal areas by employing individual condition factors and calculating the RNA/DNA ratio (Gwak and Tanaka, 2001; Smith and Buckley, 2002). However, previous studies on the early growth of larvae and seedlings comprised experiments involving the provision of artificial diets (Lauff and Hofer, 1984; Lazo et al., 2000), investigation of the effect of live food (Zambonino Infante et al., 1996; Kolkovski, 2001), improvement in early

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growth by providing exogenous hormone supplements (Kim and Brown, 1997), and assessment of nutritional requirements and reduction in malpigmentation in flounders (Rainuzzo et al., 1997; Sargent et al., 1999). The aim of these studies was to identify a beneficial somatic growth factor. But, the estimation of the final growth rate and positive somatic factors without knowledge of growth patterns is not possible to understand what the major changes during larval growth are (Zambonino Infante and Cahu, 2001; Jaravata et al., 2004; Ma et al., 2005). It is reasonable to believe that early larvae first develop the organs and tissues required for maintaining life, and that since they consume small quantities of poor quality food with low n-3 HUFA content, the digestive system is less developed during this stage. In particular, with regard to the presence of digestive enzymes, the yolk-sac larvae already possess α-amylase, and lipase (Zambonino Infante and Cahu, 2001). Moreover, despite the development of a morphological stomach, pepsin shows late activity (Kolkovski, 2001). Further, the concepts of hyperplasia and hypertrophy were introduced too sharply to the number of cells and the protein synthesis, respectively (Stoiber et al., 2002). Undoubtedly, juvenile that has similar digestion as the adults may show overlapping processes. But observing the tendency toward hyperplasia and hypertrophy is required for a precise understanding of early larval growth, because due to differences in quantity and quality of food is likely to not happen at the same time. However, studies reported the tendency toward hyperplasia/ hypertrophy during the early larval stage was rare, there are just early nutritional studies (Rainuzzo et al., 1997; Zambonino Infante and Cahu, 2001).

The olive flounder *Paralichthys olivaceus* is a commercially important species cultured in Korea and Japan (Yamashita and Yamada, 1999; Lee et al., 2000). Even though some researchers already compared the ontogeny of nucleic acids and digestive enzyme activity in growing flounder larvae, their findings tackled the early life history with nucleic acid and digestive enzymes and not the early critical periods (Gwak and Tanaka, 2001; Bolasina et al., 2005).

The aim of this study was to elucidate the early critical period through assessment of the early larval growth pattern, the content of nucleic acids changed by phosphorylations in cell, and changes in digestive enzyme activities respected by qualities and quantities of ate feed due to the observing their ecological biochemistry changes in *P. olivaceus* larvae during the rotifer feeding stages.

MATERIALS AND METHODS

Culturing of the rotifer

The rotifer *Brachionus rotundiformis* (Uljin strain in Republic of Korea) was cultured semi-continuously in 5 L plastic vessels (medium 4.5 L) by exchange of the culture medium on a daily basis (30-50%) and strong aeration (over 5 L/min) in triplicate. Moreover, the culture medium used to dilute sea water (15 ppt salinity) and maintained at a constant temperature (28 ± 0.5°C) with electric heater and dissolved oxygen was over 2 ppm. The rotifers were supplied with the condensed freshwater alga *Chlorella vulgaris* (2 mg dry matter per 2,000 rotifers) according to our previous test. Moreover the rotifer were cultured with the addition of sucrose (0.25 g of 10^6 rotifers) to enhance the activity of the digestive enzymes as a references (Yamazaki and Matsuo, 1984; Christiansen and Nielsen, 2002); in parallel a control consisting of rotifers cultured without sucrose addition was also included in the study.

Flounder larvae rearing

The flounder larvae used in the assay for the measurement of the somatic growth and activity of some digestive enzymes after the first feeding stage were obtained after they hatched from fertilized eggs (hatching rate 95%) from the Gangwon Province Marine Culture Experimental Station (GPMCES, Republic of Korea). 2,000 hatched larvae were selected and reared in three 200-L tanks (egg-shaped red rubber container, Depth 30 cm) per treatment and were maintained at 18□C under natural illumination (L/D □ 16/8, max., 3,200 lx) for 3 dph in laboratory. Larvae rearing water was exchanged 20 to 30% every other day. The treatment was to comprise either the rotifer (NR treatment, rotifer not supplied the glucose during culture with only condensed freshwater *Chlorella vulgaris*), or the rotifer cultured with an additive, that is, sucrose (0.25 g sucrose/10^6 rotifer culture, EER treatment). We added rotifers as a source of exogenous enzymes. The larvae were fed 10 rotifers/ml 2 dots a day (07:00 and 13:00) and were reared for up to 11 day post hatch (dph); on every alternate day, detritus was removed and 20% rearing seawater was exchanged with fresh seawater. Larval growth was measured in terms of length and weight to 0.01 mm and 0.002 mg, with the use of a stereo microscope (CH-30, Olympus, U.S.A.) installed scale and a balance (Mettler-Toledo, GMBH, CH/AX 26DR, U.S.A.), respectively; we randomly selected 15 larvae every morning (06:00) and subjected them to the measurements. Daily growth rates (%, DGRs) and total length/weight (TL/W) ratio that served as indicators of morphological changes in the larvae were calculated by the methods reported by Dabrowski et al. (1985) and Gisbert and Williot (1997).

Assay of nucleic acids and digestive enzymes

For the analysis of nucleic acids and the activity of digestive enzymes in the larvae and rotifers, we randomly collected 100 larvae from every tank before providing the rotifer-supplement every morning at 06:00 and 200,000 rotifers in every tank 48 h from the onset of rotifer culturing. The samples washed with distilled water, and then preserved them at -80°C. The content of the nucleic acids DNA and RNA was assayed by the spectrophotometric methods described by Fukuda et al. (1986) and Peragóna et al. (2001) and expressed as ng/larvae and the RNA/DNA ratio. The α-amylase, total alkaline protease (TAP), and triglyceride-lipase (TG-lipase) for estimation of larval digestibility were assayed with soluble starch, azocasein, and olive oil as substrates with the methods of Somogyi (1952), Kunitz (1947), and Schmidt et al. (1974). Specific activity was calculated referring the total enzyme activity to protein content estimated by the method of Lowry et al. (1951).

Statistical analysis

Linear regression ($P < 0.05$) between the larval length and the

Table 1. Digestive enzyme activities of the rotifer *Brachionus rotundiformis* cultured with/without the addition of sucrose.

Enzyme	NR	EER
α-Amylase (mU/10^6 rotifers)	32.4 ± 5.99	42.2 ± 5.63*
TAP (mU/10^6 rotifers)	8.5 ± 0.23	12.1 ± 0.40*
TG-lipase (μU/10^6 rotifers)	0.69 ± 0.024	1.10 ± 0.047*

NR and EER indicated to the normal rotifer and enzyme enhanced rotifer.*significant difference between NR and EER treatment in three digestive enzymes by paired t-test (P<0.05).

Table 2. Somatic growth (Mean±sd) and the total length/ weight ratio (TL/W, up to 11 dph) of *Paralichthys* olivaceus larvae fed with rotifer Brachionus rotundiformis cultured with/without sucrose up to 11 dph.

dph	Body length (mm)		Body weight (ug)		TL/W	
	NR	EER	NR	EER	NR	EER
3	3.21 ± 0.05^{a}	3.21 ± 0.05^{a}	52.57 ± 9.23^{cd}	52.57 ± 9.23^{ab}	0.06 ± 0.011^{a}	0.06 ± 0.011^{a}
4	3.46 ± 0.02^{b}	3.49 ± 0.04^{b}	55.00 ± 1.32^{d}	48.10 ± 9.92^{ab}	0.06 ± 0.001^{a}	0.07 ± 0.015^{ab}
5	3.62 ± 0.02^{c*}	3.71 ± 0.04^{c}	41.50 ± 5.41^{ab}	43.03 ± 2.55^{a}	0.09 ± 0.013^{bc}	0.09 ± 0.005^{b}
6	3.79 ± 0.05^{de*}	3.95 ± 0.03^{d}	49.07 ± 1.82^{bcd}	56.87 ± 5.13^{ab}	0.08 ± 0.004^{ab}	0.07 ± 0.006^{ab}
7	3.74 ± 0.07^{d*}	4.04 ± 0.09^{d}	50.10 ± 10.61^{bcd}	60.17 ± 14.54^{b}	0.08 ± 0.013^{ab}	0.07 ± 0.015^{ab}
8	3.88 ± 0.07^{ef*}	4.20 ± 0.01^{e}	38.40 ± 4.90^{a*}	58.77 ± 0.40^{ab}	0.10 ± 0.014^{c}	0.07 ± 0.001^{ab}
9	3.93 ± 0.08^{f*}	4.26 ± 0.07^{e}	41.47 ± 0.76^{ab*}	58.40 ± 12.42^{ab}	0.09 ± 0.001^{c}	0.08 ± 0.019^{ab}
10	4.00 ± 0.08^{fg*}	4.47 ± 0.08^{f}	42.07 ± 2.26^{ab*}	59.97 ± 5.20^{b}	0.10 ± 0.007^{c}	0.07 ± 0.005^{ab}
11	4.06 ± 0.09^{g*}	4.68 ± 0.10^{g}	44.00 ± 2.65^{abc*}	62.93 ± 2.91^{b}	0.09 ± 0.008^{bc}	0.07 ± 0.003^{ab}

NR and EER treatments indicate the culturing of rotifers without/with the additive (sucrose), respectively. Within columns, treatment means having a different letters in superscript within the same column are significantly different (P < 0.05). *Signify significant difference between the NR treatment and EER treatment with regard to the growth of the flounder larvae by paired t-test (P<0.05).

and ratio of the nucleic acids of larvae was calculated. The data was subjected to one-way analysis of variance (ANOVA) to test how the different treatments altered the activity of the digestive enzymes of the rotifer and somatic growth of flounder larvae with culture day. The data is presented as mean ± S.D. of 3 replicates. If significant (P < 0.05) differences were found, Duncan's multiple range test (Duncan, 1955) was used rank the groups. And we conducted to t-test (P<0.05) to compare two rotifer treatments in each day. All statistical analyses were carried out with the SPSS program version 10.0.7 (SPSS Inc., 2000, Michigan Avenue, Chicago, Illinois, U.S.A.).

RESULTS

Three digestive enzymes activities of two sort rotifers, *B. rotundiformis* used in this study were shown in Table 1. Activities of α-amylase, TAP and TG-lipase were higher assayed in EER treatment than that of NR treatment (P<0.05).

Length, weight and TL/W ratio up to 11 dph of flounder larvae fed two sort rotifers were shown in Table 2. Total length significantly differed between treatments from 5 dph until 11 dph and constantly increased. Lengths were significantly differed with NR treatment, 4.06±0.09 mm, and EER treatment, 4.68±0.10 mm, respectively (P<0.05). Weight was increased up to 7 dph in EER treatment, but it was not significantly differed with the previous day (P<0.05). But the weight was constantly increased after decrease on 8 dph. Body weight of NR treatment decreased from 52.57 μg pm 3 dph to 38.40±4.90 ug/larva on 8 dph (P>0.05), while increased up to 44.0±2.65 μg/larva on 11 dph. On the other hand, EER treatment was not shown the significant decrease (P>0.05), and the treatment was significantly differed with 44.0±2.65 ug/larva of NR treatment to 62.93±2.91 ug/larva on 11 dph (P<0.05). TL/W ratio was significantly decreased on 6, 7 dph in NR treatment, whereas EER treatment was not shown the significant decrease (P>0.05). And EER treatment showed the lower TL/W ratio than that of NR treatment after 6 dph.

Two treatment larval daily growth rate (DGR) of length and weight was shown in Figure 1. Length and weight DGR had twice decreasing periods. The decreasing periods of length DGR showed on 7 and 9 dph, especially in NR treatment showed the (-) DGR on 7 dph. In the case of weight DGR, it showed the decreasing periods on 5 and 8 dph, and the DGR was shown bigger changes until (-) value in NR treatment than that of EER treatment. And the DAG of both treatments appeared (-) values on 5 dph, NR treatment showed lower (-) DGR than that of EER treatment on 8 dph, especially.

Variations of nucleic acids by length growing of two sort flounder larvae were shown in Figure 2. The relationship

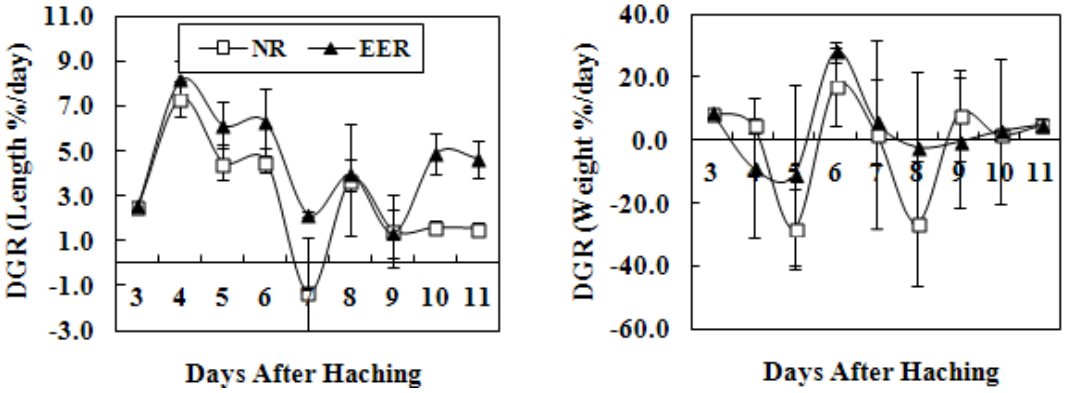

Figure 1. Daily growth rate (DGR, %/day) of the length and body weight (DW) of *Paralichthys olivaceus* larvae fed with rotifer, *Brachionus rotundiformis* cultured with/without sucrose up to 11 dph. In the legend NR (□) and EER (▲) treatments were same as Table 2.

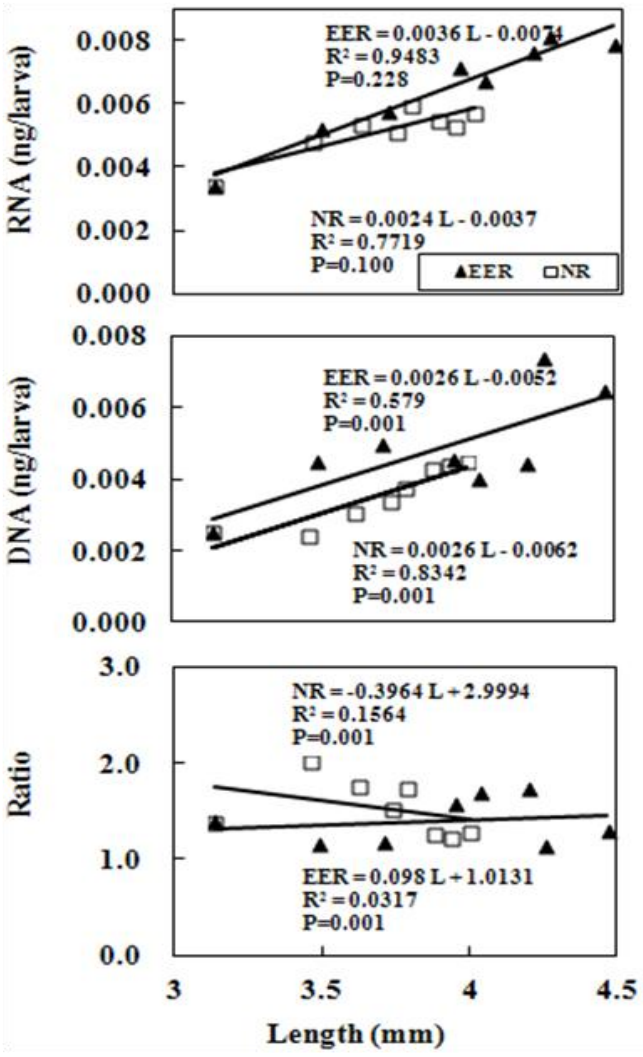

Figure 2. Relationship between the DNA, RNA, and their ratio and length (mm) of Paralichthys olivaceus larvae (up to 11 dph) fed with rotifer Brachionus rotundiformis cultured with/without sucrose up to 11 dph. NR (□) and EER (▲) treatments are the same as those in Figure 1.

with larval length and RNA content showed tendency increased with all length growth, but not significantly differed (EER treatment, EER=0.0036 L – 0.0074, R^2=0.9483, P=0.228; NR treatment, NR=0.0026 L – 0.0037, R^2=0.7719, P=0.100; P>0.05). But RNA content of NR treatment showed with low length growth. Relationship with length and DNA content significantly increased (both tendency of NR and EER treatment is shown P<0.05), and low DNA content of NR treatment was shown with low length growth. And RNA/DNA ratios of both treatments were shown the significantly different increasing (P=0.001) and decreasing (P=0.001) tendency in EER treatment and NR treatment, respectively. And α-amylase, total alkaline protease and TG-lipase of two sort flounder larvae were shown to specific activities (mU/mg protein) on 3, 7 and 11 dph in Figure 3. α-Amylase and TG-lipase activities were not significantly differed on different each day, while total alkaline protease activities of both treatments were shown the significant difference on 7 dph. And the activities of NR treatment showed the lowest activity, 44.9±47.68 mU/mg protein on 7 dph and the highest activity, 413.7±196.85 mU/mg protein on 11 dph, respectively (P<0.05).

DISCUSSION

In the fish larvae, critical periods occur during the early life cycle due to several physiological constraints (Hjort, 1914; Cushing, 1969, 1990; Kim and Brown, 1999; Voss et al., 2001). During the critical period, larvae die before/after first feeding due to (1) the lack of essential nutrients in the yolk sac due to poor condition of brood stock nutrients before the initiation of exogenous feeding, (2) poor digestion during the early exogenous feeding period, and (3) inadequate supply of nutrients in the diet (live food). Further, the hatching rate of fertilized eggs is reduced due to malnutrition of the broodstock. In addition, in the artificial rearing process, early larvae go through an unnatural critical period due to consumption of diets

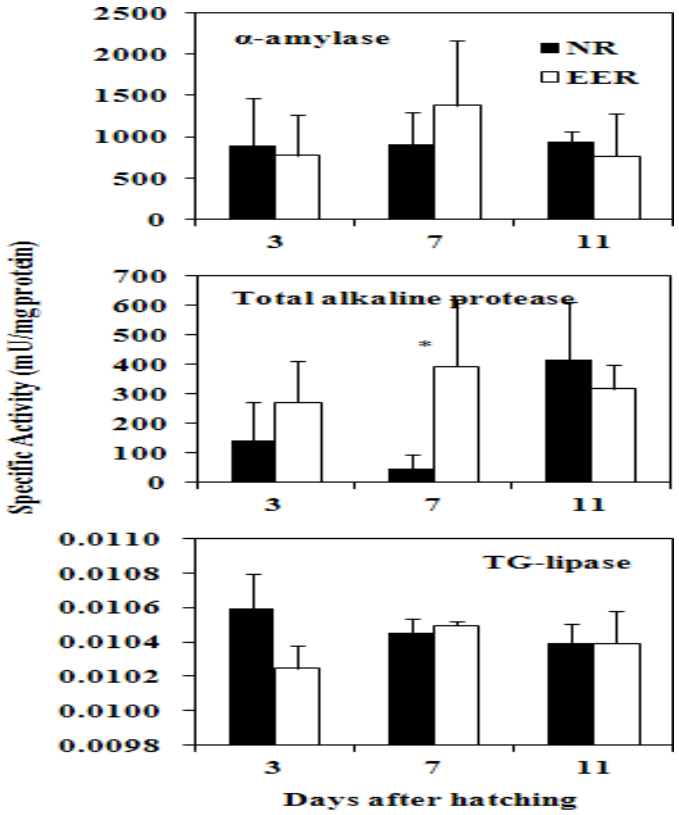

Figure 3. The α-amylase, total alkaline protease and triglyceride-lipase (TG-lipase) activity at 3, 7, and 11 dph of the flounder (*Paralichthys olivaceus*) larvae (up to 11 dph) fed with rotifer *B. rotundiformis* cultured with/ without sucrose up to 11 dph. *significant difference by the *t* test ($P < 0.05$). The NR and EER treatments are the same as those in Figure 1. The different superscripts as a, b, c were indicated the significant difference on the different days post hatch.

supplemented with artificial microparticles before complete development of the digestive system.

We fed the larvae with rotifers that sucrose could enhance the activity of some digestive enzymes in the rotifers by 30.2 to 59.4% (Table 1). However, despite the use of rotifers with artificially enhanced digestive enzyme activity, we could not prevent the decrease of larval weight at 5 dph, but observed a gradually increase in somatic growth at 7 dph ($P<0.05$) (Figure 1); we attributed this surge in growth to the development of digestive organs, in particular, the intestines, because the stomach had not yet developed at the rotifer-feeding phase (Kurokawa and Suzuki, 1996). Therefore, though a survival rate of the larvae who initial poor digestive function did not confirm, we suggest that rotifer supplementation enhanced digestive enzyme activities to larvae during the critical period (Table 1).

Olive flounder larvae reared on a diet of either of the 2 types of rotifers (cultured normally or with the addition of sucrose) was shown in Table 2. The larval weight-growth rate decreased initially at 5 to 6 dph (the first growth-rate-reduction period), and this decrease was independent of the type of diet. In contrast, the change in pattern occurring at 8 to 9 dph (the second growth-rate-reduction period) involved a decrease in larval weight (in Table 2, a decrease is reported); Thus, the first growth reduction period, which was independent on the type of diet, was probably due to the adaptation of the larvae to the exogenous diets, while the second critical period was attributed to the poor quality of the diet. In the NR treatment in which only condensed freshwater *Chlorella* sp. was supplied, we observed a gradual increase in body length up to 7 dph, but the weight decreased at 5 to 6 dph and 8 to 9 dph. In contrast, in the EER treatment in which an appropriate nutritional diet (rotifers containing more digestive enzymes) was supplied, constant growth without any decrease in larval length was observed during the abovementioned period, but the weight decreased at just 5 to 6 dph. So to enhance activities (that is, digestive enzymes) of food should be benefit for early flounder larvae.

The TL/W ratio was reported to be an important index of morphological variations in fish larvae (Gisbert and Williot, 1997). On the basis of this index, considering the body length and weight patterns in the NR treatment, the growth pattern showed once reduction in the TL/W ratio at 6 to 7 (Table 2). In the EER treatment, the larvae initially appeared to show decreased growth, similarly that observed in the NR treatment, but the decrease was smaller than that in the NR treatment (Figure 1). The critical periods of growth corresponded with the critical periods reported by Voss et al. (2001), who suggested that the phenomenon was caused by the early phase of maladaptation and inadequate supply of nutrients in exogenous diets.

Both the treatments showed the first reduction in the DGRs, and this reduction coincided with the first critical period, that is, the adaptation phase of the larvae to exogenous diets after the first feeding (Figure 1). Thereafter, the larvae in the NR treatment again showed a significant reduction in the DGRs at 4 to 5 dph (Figure 1); the reason for this reduction might be related with the low activities of total protease on 3 and 7 dph as period with first feeding and starting developing and adapting of some digestibility (Figure 3). The weight/length ratio decreased as a consequence of the weight loss due to periodic differences of hypotrophy and hypoplasia. In contrast, the larvae in the EER treatment showed a steady increase in weight after the first critical period (Figure 1). On the basis of this observation, we think that (1) lipid enrichment is required to achieve a steady increase in body length and weight in the indicated period, and that (2) a further increase in the weight of the larvae should not be expected when rotifers are cultured only with the addition of sucrose, without any lipid enrichment. The standard weight- and length-daily growth patterns observed in the larvae were as follows. The body length increased constantly, reaching the peak of length-

daily growth, while the weight decreased, showing a negative weight-daily growth rate. Thereafter, the weight-daily growth rate peaked at 6 to 7 dph, following which it decreased.

Moreover, there was a period during which the decrease in weight was not due to the lack of nutrients in exogenous diets since it was also observed in the EER treatment supplemented with sucrose. In that period, the body length-daily growth rate was opposite that of the weight-daily growth rate since the latter decreased significantly at 7 dph (Figure 1).

The DGRs of the flounder larvae, which were fed on the 2 types of rotifers, tended to show similar growth patterns. However, the reduction in the DGRs was more significant in the NR treatment than in the EER treatment, and the period from 4 to 6 dph was considered as the first critical period since it showed a negative weight DGR after the first feeding. The survived larvae exhibited an increase in body length due to their own enhanced digestion after this critical period, but the body weight in these larvae decreased at 8 dph was not evident because further growth was not possible due to the lack of nutrition after the first critical period (Table 2). One possible reason for this tendency may be that nutrients which support the functions of the developed larval digestive organs (that is, the intestines) were not supplied.

The growth patterns of the larvae supplied of either of the 2 types of rotifers at 8 dph, the second critical period after the first feeding were similar with respect to variations in body weight; however, the length-daily growth rate was high in the EER treatment (Figure 1). The larvae in the NR treatment did not demonstrate hyperplasia and hypertrophy in the indicated period. Meanwhile, the larvae in the EER treatment simply demonstrated hyperplasia because only an increase in length was observed. As observed in this study, somatic weight did not increase even after the larvae were supplemented with the 2 types of rotifers. The rotifers supplied to the larvae lacked essential components (that is, nutrients as DHA and EPA) probably because the rotifers assimilated quite a few nutrients and a considerable amount of the digestive enzymes; therefore, weight of the larvae was not increased. Therefore, we suggest that lipid- and digestive enzyme-enriched rotifers are required for early increase in body weight.

NR treatment presented lower increase in RNA content, which decreased further and eventually become lower than those in the EER treatment in larvae measuring over 3.8 mm (Figure 2). The predictions of lower RNA contents in NR lowered the protein metabolism treatment were consistent with the real data because the low RNA contents in cells. Moreover, 3.2 mm long larvae exhibited length growth, that is, hyperplasia due to increase in the DNA content and cell differentiation. However, in the 3.4 to 4.2 mm larvae, an increase in the cell size or the quantity of cytoplasm, that is, hypertrophy, was suggested because only the RNA content increased in EER treatment. Unlike the larvae in the EER treatment, the larvae in the NR treatment did not show somatic length growth of more than 4.0 mm. Due to the low RNA contents, this observation could be suggested to various aspects of nutritional digestion and absorption. It is currently difficult to provide a more detailed explanation for a result like the abovementioned one; it could have been explained hypothesizing that numerous growth hormones were expressed in larvae with high DGRs and in larvae supplied with good-quality diets (Ohlsson et al., 1998; Ariznavaeerta et al., 2003). Larvae measuring more than 4.0 mm in length were not obtained in the NR treatment and the RNA content, which is related to intracellular protein synthesis, decreased compared to EER treatment; on the basis of this, we consider the rotifers supplied immediately after culturing to be an inadequate source of nourishment. The consequent lack of nutrition and decreased RNA content led to reduced cell differentiation. Additionally, considering the RNA/DNA ratio in the critical periods, in the case of a high ratio of 1.3 in the 3.2- to 3.8-mm larvae, a further increase in value is not desirable because poor cell differentiation is related with the DNA content rather than the RNA produced in the length growth phase. Moreover, despite the high production of RNA in the EER treatment, we predicted that the RNA/ DNA ratio later decreases, on the basis of the observation that in the EER treatment, the larvae consumed a relatively rich diet and had a high DNA content. During the period of the significant increase in the body weight of larvae measuring 3.8 to 4.2 mm in the NR treatment, the RNA/DNA ratio was lower than 1.3, because there was no increase in the DNA content and RNA synthesis. This leads to the conclusion that in the NR treatment, DNA synthesis exceeded RNA synthesis, because in this period, growth in length rather than in weight was observed (Figure 2).

Digestive enzyme activities developed as a consequence of the development of digestive organs, which occurs during the somatic growth. Further, we investigated the changes in the growth patterns and digestive enzyme activities in the larvae reared by supplying 2 types of diets. We found that α-amylase, TAP, and TG-lipase activities did not differ significantly between the larvae in the NR and EER treatments (with the exception of TAP at 7 dph) ($P < 0.05$) (Figure 3). The investigation of larval growth patterns and digestive enzyme activities by administration of different diets revealed that the activities varied depending on the digestive organs developed. Further, the secretion of digestive enzymes within the internal organs changed abruptly over time. Thus, we may conclude that *P. olivaceus,* larvae underwent 2 critical periods during the rotifer-feeding stage after the first feeding. We think that the first critical period corresponds to the adaptation of the larvae to exogenous diets and the second critical period, to the lack of adequate nutrients in these diets.

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