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## **Survival, Growth and RNA/DNA Ratio of *Pagrus major* Cultured under Three Different Feeding Regimes During Early Development**

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### **Abstract**

The nutritional status of red sea bream *Pagrus major* (30-day-old) cultured under three different feeding regimes: 1) rotifer, *Artemia* and artificial diet (RAA), 2) rotifer and artificial diet (RA) and 3) artificial diet (A) only were evaluated depending on RNA/DNA ratios. The duration of the experiment was four weeks. The final average weight of fish was significantly ( $P < 0.05$ ) higher in RAA ( $1555 \pm 119$  mg) than RA ( $1010 \pm 145$  mg) and A ( $927 \pm 170$  mg). Specific growth rate was significantly ( $P < 0.05$ ) higher in RAA (7.376) and RA (5.617) at the end of the first week of culture compared to the other weeks. In artificial diet fed fish, SGR was significantly ( $P < 0.05$ ) higher at the end of the second week (4.542) compared to the other weeks. The DNA concentration was significantly ( $P < 0.05$ ) higher at the end of the second week of feeding than in the remaining culture period regardless of feeding conditions. The RNA concentration increased from

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the first to the second week of culture, followed by a decrease in RNA concentration at the end of the third week and then a re-increase at the end of the fourth week in three treatments. The amount of RNA of fish was significantly ( $P < 0.05$ ) higher in RAA and RA treatments than in artificial diet fed fish at the end of the second week. The RNA/DNA ratio showed a direct relationship with growth rate in these three different treatments. The RNA/DNA ratio was significantly ( $P < 0.05$ ) higher in RAA than RA and A showing the superiority of this feeding regime during early development.

## Introduction

Red sea bream *Pagrus major* (Class: Actinopterygii, Order: Perciformes, Family: Sparidae) is an important commercial fish in Japan. The aquaculture production of red sea bream occupies the second largest position in Japan (Koshio 2002). Mass mortality in 1 and 2 yr old *P. major* has been reported during winter season with the symptoms of severe anemia (Miyazaki et al. 2000). Understanding of the nutritional status of this fish is essential during ontogenesis for the development of efficient rearing technique. The growth of fish depends largely on protein energy (Koshio 2002). The RNA serves as both a template and an organizer for protein synthesis, and thus, total RNA varies directly with protein synthetic activity and is expected to show an increase in tissues undergoing accelerated growth (Bulow 1987). The RNA content has been proposed as an instantaneous index of somatic growth and nutritional status in fish (Houlihan et al. 1993; Bergeron 1997). The amount of DNA, the carrier of the genetic information, is constant in somatic tissues and tissue concentrations reflect cell numbers (Dortch et al. 1983). The relationship between RNA and DNA is an index of the cell's metabolic intensity and has been used to measure growth of fish (Bulow 1987). The ratio of RNA/DNA is a useful indicator of nutritional condition. The RNA/DNA ratio can reflect food availability and food quality, but is not affected by food in the gut (Clemmesen 1996). Since larval growth is dependent upon protein synthesis, the RNA/DNA ratio has been shown to be highly sensitive to feeding levels and can be used as an index of fish growth (Gwak and Tanaka 2001). Kimura et al. (1996), Gwak and Tanaka (2001, 2002) have studied the RNA/DNA ratio of sardine *Sardinops melanostictus* and Japanese flounder *Paralichthys olivaceus* larvae, respectively to understand their nutritional conditions. Live food plays an important role in larviculture, but the replacement of live food by artificial diet is essential to make aquaculture commercially feasible. Usually the red sea bream larvae are fed with smaller rotifers at first feeding and gradually shifted to larger live food like

*Artemia* sp. and finally to artificial diet. But live food may play a vital role even during late larval stages. The aim of this study is to evaluate the effects of three different feeding regimes of rotifer, *Artemia* and artificial diet, rotifer and artificial diet and artificial diet only on the nutritional status of sea bream *P. major* larvae based on RNA/DNA ratio under cultured conditions. This study helps to understand the importance of live food even during the late developmental stages of *P. major*.

## Materials and Methods

### *Larval rearing*

The fertilized eggs of red sea bream *Pagrus major* were collected from the Kyoto Prefectural Sea-Farming Center and were kept in a flow through system (500-L tank) at an ambient temperature of 23°C at the Fisheries Research Station of Kyoto University, Maizuru. Hatchlings were obtained within 24 h. Larvae were grown on the same diet for 30 days post-hatching before the experiment commenced. Exogenous feeding with rotifer *Brachionus plicatilis* was started from the second day and larger food like *Artemia* and commercially available artificial diet (Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan) were fed at 5% of body weight from the 10th day onwards. Fish were cultured under three different feeding regimes from the 30th day onwards. In the first treatment (RAA), larvae were fed with a mix diet of rotifer *Brachionus plicatilis*, *Artemia* and artificial diet; in the second treatment, rotifer *B. plicatilis* and artificial diet and in the third one (A), only artificial diet (Table 1). Live food *B. plicatilis* and *Artemia* were enriched with DHA oil. Three replicates were used for each feeding scheme. Stocking density was 50 fish/100-L tank. Two particle sizes of artificial diet 400 µm and 700 µm were used in this study. Artificial diet was given in two installments, the first at 9 a.m. and the second at 6 p.m. Rotifers and *Artemia* were given at 1.30 p.m. and 4.30 p.m. Duration of the experiment was four weeks.

### *Sampling and weight measurement*

Sampling was performed on the 30th day, at the start of the experiment and at weekly intervals (15 fish for each feeding scheme, five fish from each culture tank) before feeding. Wet weight of individual fish was measured.

Table 1. Feeding schedule of red sea bream *P. major* under three experimental conditions during 28 days of culture period

	RAA	RA	A
Rotifer (no. tank <sup>-1</sup> )	30,000	30,000	-
<i>Artemia</i> (no. tank <sup>-1</sup> )	3,000	-	-
Artificial diet (mg tank <sup>-1</sup> )			
400 µm	200	200	200
700 µm	1,000	1,000	1000

RAA = Rotifer, *Artemia* and Artificial diet fed group, RA = Rotifer and Artificial diet fed group, A = Artificial diet fed group.

### ***RNA and DNA estimations***

Same fish collected for weight study were cleaned thrice in distilled water to remove impurities, kept in tubes, immediately frozen at -25°C and stored at -87°C for biochemical assays. The RNA and DNA contents of the whole body of individual fish were measured. The RNA and DNA were determined by the fluorescence technique using ethidium bromide (Nacalai Tesque Co. Ltd., Kyoto, Japan), following [Clemmesen \(1993\)](#), as modified by [Sato et al. \(1995\)](#). Salmon sperm DNA (Wako Chemicals, Osaka, Japan) and yeast RNA (Kanto Chemicals, Tokyo, Japan) were used as standards. Both RNA and DNA are expressed as µg mg<sup>-1</sup> wet weight of fish.

The specific growth rate (SGR) was calculated using the formula:

$$\text{SGR} = 100(\ln W_t - \ln W_i)/t$$

Where  $W_i$  and  $W_t$  are the initial and final body weights and  $t$  the time in days.

Differences in fish growth, RNA and DNA concentrations were assessed by one-way ANOVA, Duncan's multiple range test, DMR ([Montgomery 1984](#)) and regression analysis. The level of significance was  $P < 0.05$ .

## Results and Discussion

There was no significant ( $P > 0.05$ ) difference for average weight ( $74 \pm 2.56$  mg), RNA ( $0.95 \pm 0.08 \mu\text{g mg wet weight}^{-1}$ ) and DNA ( $0.76 \pm 0.11 \mu\text{g mg wet weight}^{-1}$ ) concentrations of red sea bream at the beginning of the study (0 time point). The weight of red sea bream increased during 28 days rearing period in all these three treatments. The final average weight of fish was significantly ( $P < 0.05$ ) higher in the first treatment, RAA ( $1555 \pm 119$  mg) than the second treatment, RA ( $1010 \pm 145$  mg) and the third treatment, A ( $927 \pm 170$  mg). In RAA treatment, the average weight of fish was 7.87 % (second week) to 41.69 % (third week) higher than RA and A (Fig.1). Specific growth rate (SGR) was significantly ( $P < 0.05$ ) higher in RAA ( $7.376 \pm 0.06$ ) and RA ( $5.617 \pm 0.04$ ) at the end of the first week of culture than in other weeks. In artificial diet fed fish, SGR was significantly ( $P < 0.05$ ) higher at the end of the second week ( $4.542 \pm 0.02$ ) than the first, third and fourth weeks (Table 2). The first two weeks might be a period of adjustment to artificial diet fed fish and this resulted in delayed growth rate. There was no mortality of fish throughout the study period.

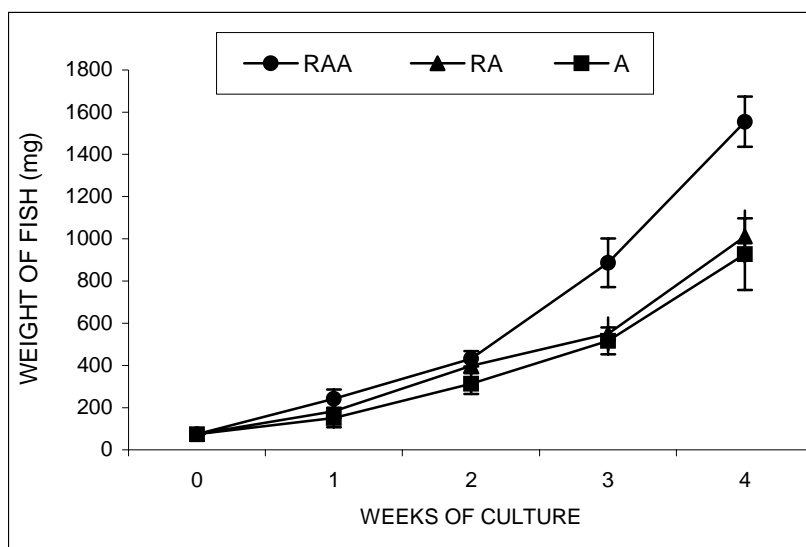


Fig. 1. Fresh weight of *P. major* cultured under three different feeding regimes during four weeks of culture (n=15, bars = SEM)

Table 2. Specific growth rate of red sea bream *P. major* during 28 days of rearing under three different feeding schemes of RAA (Rotifer, *Artemia* and Artificial diet), RA (Rotifer and Artificial diet) and A (Artificial diet)

Feeding scheme	First week	Second week	Third week	Fourth week
RAA	7.376±0.06 <sup>a</sup>	3.570±0.03 <sup>c</sup>	4.456±0.004 <sup>b</sup>	3.490±0.01 <sup>c</sup>
RA	5.617±0.04 <sup>a</sup>	4.820±0.03 <sup>b</sup>	2.007±0.01 <sup>d</sup>	3.771±0.007 <sup>c</sup>
A	4.424±0.07 <sup>b</sup>	4.542±0.02 <sup>a</sup>	3.094±0.03 <sup>d</sup>	3.623±0.01 <sup>c</sup>

Means not sharing the same superscripts are significantly different ( $P < 0.05$ ).

The DNA concentration was higher at the end of the second week of feeding than in the remaining culture period in all these three treatments (Fig. 2). Among these three feeding conditions, DNA concentration was significantly ( $P < 0.05$ ) higher in RAA compared to the other two treatments in the first, third and fourth weeks of sampling. In a weight gaining fish, the DNA content becomes diluted with a larger volume of cells per unit weight (Behanan and Mathew 2004). In the present study, the average weight of fish increased with age of fish, consequently resulting into low DNA content.

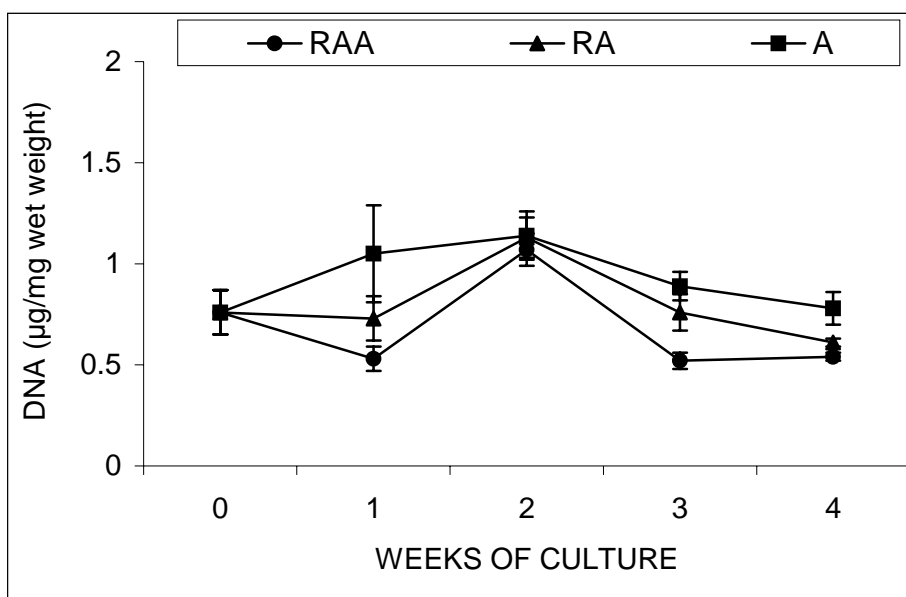


Fig. 2. Developmental changes in DNA content of *P. major* cultured under three different feeding regimes during four weeks of culture (n=15, bars = SEM)

In all these three treatments, RNA concentration increased from the first to the second week of culture, followed by a decrease of RNA concentration at the end of the third week and then a re-increase at the end of the fourth week (Fig. 3). The amount of RNA of fish was significantly ( $P < 0.05$ ) higher in RAA and RA treatments compared to the fish fed with only artificial diet at the end of the second week. There was no significant ( $P > 0.05$ ) difference between RAA and RA treatments at this time. Ontogenic changes in DNA and RNA contents have been recorded in Japanese flounder *Paralichthys olivaceus* (Gwak and Tanaka 2002).

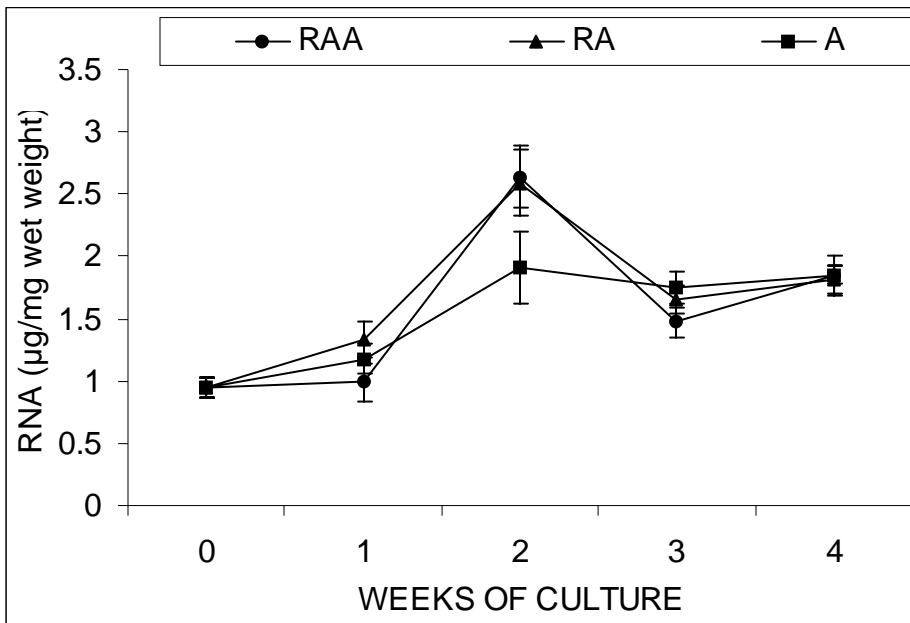


Fig. 3. Developmental changes in RNA content of *P. major* cultured under three different feeding regimes during four weeks of culture (n=15, bars = SEM)

The RNA/DNA pattern of juvenile *Solea solea* and *Solea senegalensis* reflected growth (Fonseca et al. 2006). Imsland et al. (2002) observed a positive and almost significant correlation between mean growth rate and the corresponding RNA/DNA ratio in the white muscle of juvenile turbot *Scophthalmus maximus* (L.). In the present study, RNA/DNA ratio also showed direct relationship with growth rate (Fig. 4) in RAA ( $y = 0.463x + 1.099$ ,  $R^2 = 0.9904$ ), RA ( $y = 0.341x + 1.241$ ,  $R^2 = 0.9522$ ) and in A ( $y = 0.26x + 1.064$ ,  $R^2 = 0.7625$ ) treatments. The RNA/DNA ratio showed a gradual increasing trend from the start of feeding trial to the end

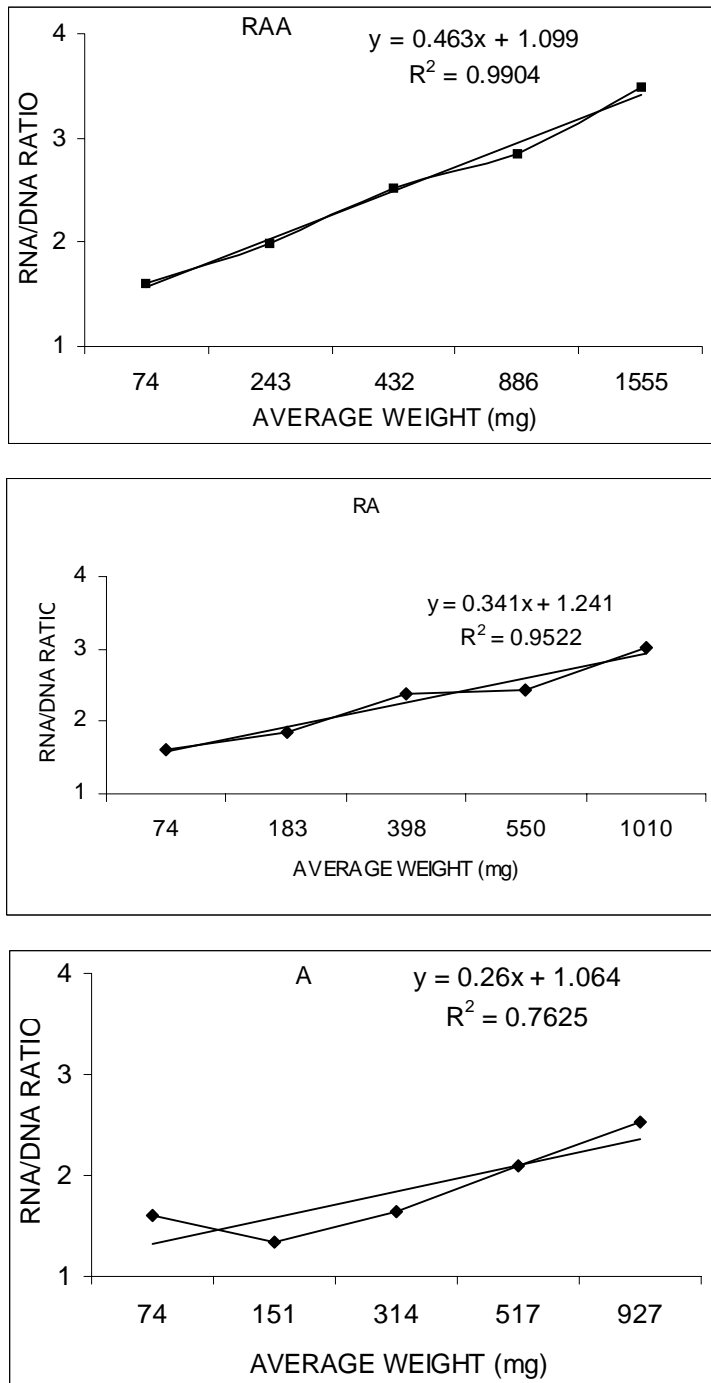


Fig. 4. Relationship between average weight and RNA/DNA ratio of *P. major* cultured under three different feeding regimes.



of the fourth week in these treatments (Fig. 5). Since the DNA content of a cell is normally constant, measurements can be made of fluctuations in cell numbers and cell divisions in an organism. The DNA is a prerequisite for RNA synthesis, which in turn is a requirement for protein synthesis (Raece et al. 1988). Obviously the ratio of RNA to DNA, and protein to DNA, will reflect the cellular ability to produce RNA and proteins, and hence these parameters measure the potential for growth. The decrease in RNA/DNA in a certain tissue can be attributed to reduced protein synthesis or increased DNA content by cell division (hyperplasia). On the other hand, increments in cell size produce higher RNA/DNA since the amount of DNA is constant, i.e. hypertrophy (Lemos et al. 2002). In the present study, RNA/DNA ratio increased at first and second weeks due to the increase in the amount of RNA. In the following third and fourth weeks, the amount of DNA decreased resulting in a higher RNA/DNA ratio. The RNA/DNA ratio was always higher in RAA (1.98 to 3.49) treatment than RA (1.86 to 3.03) and A (1.34 to 2.53). Kimura et al. (1996) have diagnosed the nutritional status of larvae of Japanese sardine *Sardinops melanostictus* on the basis of RNA/DNA ratio by the following criteria: healthy fish had RNA/DNA ratios greater than or equal to 1.48, poor condition was reflected by ratios between 1.48 and 1.32 and starving fish had ratios of less than or equal to 1.32. The mean RNA/DNA ratios in fed Japanese sardine *Sardinops melanostictus* (Kimura et al. 1996), herring *Clupea harengus* (Clemmesen 1994) and Dover sole *Solea solea* (Richard et al. 1991) ranged from 1.6 to 2.8, 1.6 to 4.5 and 1.5 to 2.3, respectively during the first 15 days after hatching. Westerman and Holt (1994) demonstrated that a RNA/DNA ratio of approximately "2" is regarded as "minimum threshold ratio" and indicated the threshold level of protein synthesis capacity required for normal development and growth. In the present investigation, RNA/DNA ratio ranged from 1.98 to 3.49, 1.86 to 3.03 and 1.34 to 2.53 in RAA, RA and A treatments, respectively. In rotifer, *Artemia* and artificial diet fed fish, the values were above the "minimum threshold ratio", which was suggested by an earlier researcher (Westerman and Holt 1994). This reflects the superiority of mixture of live food (rotifer and *Artemia*) and artificial diet compared to rotifer and artificial diet fed group and only artificial diet fed group.

It has been suggested that the live food organisms consumed by larvae assist the digestion process by 'donating' their digestive enzymes, either by autolysis or as zymogens that activate the larval endogenous digestive enzymes. Live food organisms also contain gut neuropeptides and nutritional "growth" factors which enhance digestion. These sub-

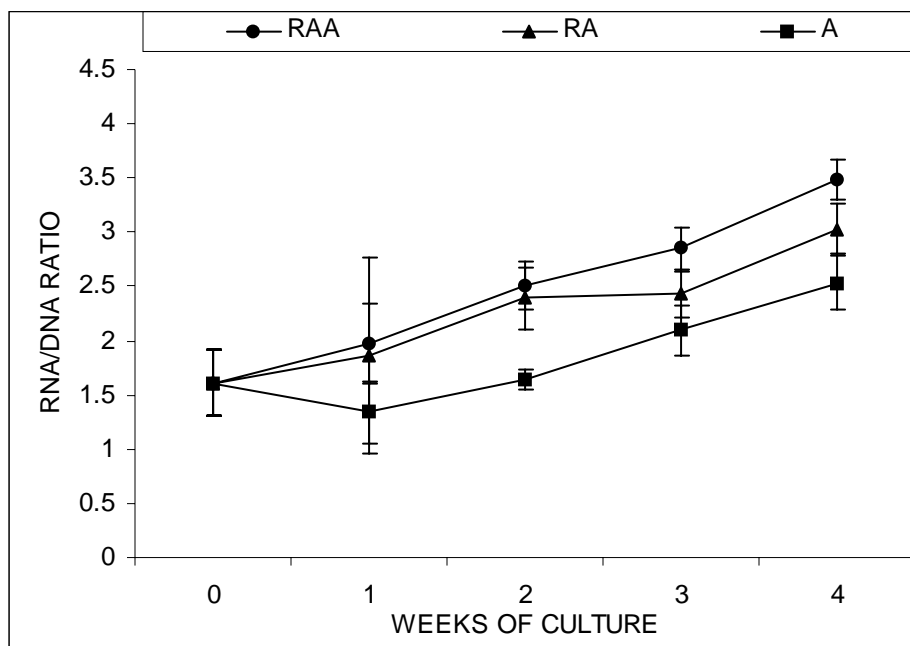


Fig. 5. Developmental changes in RNA/DNA ratio of *P. major* cultured under three different feeding regimes during four weeks of culture (n=15, bars = SEM)

stances are frequently omitted in formulated diets (Kolkovski 2001). Since larvae swallow their prey, they are usually alive in the oesophagus. The movement of the live organisms may cause movement of the gut walls and the microvilli, stimulating neuropeptides release (Kolkovski 2001). Moreover, particulate diets for larvae contain proteins and other ingredients that are difficult to digest (formulated diets contain 60-90% dry matter, while zooplankton only 10%). Cahu et al. (1998) observed that survival and final weights of sea bass *Dicentrarchus labrax* larvae were higher in groups fed live prey than in groups fed the compound diet, though larvae really ingested the microparticulated diet. The average weight of common carp *Cyprinus carpio* was 3- to 5-fold higher in fish fed with live food compared to artificial diet fed one (Sharma and Chakrabarti 1999). In the present study, better performance of *P. major* was found in fish fed with mixture of rotifer, *Artemia* and artificial diet compared to the other two groups. This also shows the importance of supply of *Artemia* and rotifer along with artificial diet in the feeding schedule of *P. major* even after 30 days of hatching. This information may be used for the culture of red sea bream.

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