EFFECTS OF FEEDING ON THE GROWTH AND SURVIVAL OF RED SEABREAM LARVAE *Pagrus major*.

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RESUMO

O presente experimento foi conduzido no laboratório do Centro Internacional de Treinamento Pesqueiro de Kanagawa - Japão, durante 22 dias, entre 27 de abril e 19 de maio de 1990, com o objetivo de observar o desenvolvimento larval do "RED SEABREAM" *Pagrus major*, em comprimento total (TL), altura do corpo (BD) e a taxa de sobrevivência, administrando-se 3 diferentes dietas alimentares. No tratamento 1, utilizou-se o rotífero alimentado com a "chlorella" marinha (*Nannochloropsis oculata*), no tratamento 2, o rotífero alimentado com uma mistura de "chlorella" marinha e fermento e no tratamento 3, o rotífero alimentado com fermento e enriquecido pelo método direto (lipídios emulsionados). Os dados obtidos ao final do experimento foram tabulados e traçadas as curvas de crescimento em TL e BD. Os resultados para os tratamentos 1, 2 e 3 foram 7,84mm ± 0,04 & 1,94mm ± 0,31, 7,63mm ± 1,11 & 1,88mm ± 0,68 e 7,08mm ± 0,58 & 1,74mm ± 0,17, em TL e BD, respectivamente e as taxas de sobrevivência foram 67,8% ± 8,17, 69,6% ± 6,99 e 70,3% ± 6,95, respectivamente. Os resultados mostraram que nenhuma diferença significativa foi encontrada entre os tratamentos, através da análise estatística de variância (ANOVA).

ABSTRACT

An experiment was conducted for 22 days, from April 27 to May 19, to observe the larval development of "RED SEABREAM" *Pagrus*

major in total lenght (TL), body depth (BD) and the survival rate, fed on three different diets. Treatment 1 was rotifers fed on marine "chlorella" (*Nannochloropsis oculata*), treatment 2, rotifers fed a mixture of marine "chlorella" and baker's yeast and treatment 3, rotifers fed on baker's yeast enriched by the direct method (emulsified lipids). The data obtained at the end of the experiment was computed in tables and growing curves in TL and BD were drawn. The results for treatment 1, 2 and 3 were, 7.84mm ± 0.04 & 1.94mm ± 0,31, 7.63mm ± 1.11 & 1.88mm ± 0.68 and 7.08mm ± 0.58 & 1.74mm ± 0.17, in TL and BD, respectivelly and the survival rate was 67.8% ± 8.17, 69.6% ± 6.99 and 70.3% ± 6.95, respectivelly. The results showed that no significative difference was found between treatments by statistical analysis of variance (ANOVA).

INTRODUCTION

The larval rearing of marine finfish are being developed worldwide, due to its economical and nutritional importance for human being. Therefore, to achieve better profits in growout aquaculture activities, it is showing to be very important the whole control of eggs and larvae stages, with special attention to the carefull selection of healthy broodstock, which will be the initial stage of larval rearing.

Red seabream *Pagrus major* is one of the most important specie for mariculture in Japan and, during the past decades, extended research have been directed toward an improvement in rearing techniques for early life history of this specie. The principal reason to achieve the feasibility of mass production of red seabream fries was the success use of rotifer *Brachionus plicatilis*, a brackish zooplankton, as the feed to raising the larvae and juveniles of this fish (FUKUSHO, 1987).

The reproduction and egg quality of red seabream, were found to be deeply affected by nutritional quality of diet for broodstock (WATANABE, 1984). The quality of eggs will directly affect the larvae development, showing that a nutritional value diet is very important for the development of marine fish, mainly related with essential fatty acids (EFA) required by the specie to grow and to develop its reproductive life. There are few studies on the EFA requirement of red seabream larvae, but it is known that EFA requirement differ not only between species but also between growth stages (IZQUIERDO et al., 1989). According IZQUIERDO et al. (1989), the minimum EFA requirement for red seabream larvae is about 3.46% in dry basis of rotifers. The high mortality observed in many fish larvae during feeding with live food organisms are, sometimes, due to an EFA defficiency in the fish (IZQUIERDO et al., 1989).

A number of live food organisms has been utilized and experienced for the primary feed of larvae as rotifer, *Artemia*, copepod and the trochophore larvae of oyster. Rotifers have been the most important live food for the early days of larvae, while enriched during their culture. Experiment have shown that larvae fed on rotifer containing a low percentage of n-3 HUFA (High Unsaturated Fatty Acid), showed a poor growth and a high mortality (IZQUIERDO et al., 1989). Nevertheless, as said above, rotifer can be enriched by different methods to ensure the require necessity of EFA of each specie. One of the most common method utilized is the culture of rotifer fed with the so called marine "chlorella" (*Nannochloropsis oculata*), being used both by governmental and private sectors, due to its handling facility, specially in large scale system. Other methods utilized are: the indirect method, when a newly developed yeast (w-yeast) is utilized and the direct method, when rotifers are fed baker's yeast and enriched by emulsified lipids (emulsion oil).

The objectives of the present experiment were to test three differents feeding treatments in the rearing of red seabream larvae, during a period of 22 days, from April 27 to May 19, 1990, when the daily larval development stages were observed. Measurement of total lenght (TL), body depth (BD), survival rate at the end of the experiment and the mortality at two differents stages, were used to compare the effects of the 3 treatments utilized.

MATERIALS AND METHODS

Fertilized eggs of red seabream *Pagrus major*, were obtained from the Kanagawa Prefectural Fishery Experimental Station where adults of red seabream naturally spawn in tanks. The eggs were transported to the Kanagawa International Fishery Training Center Laboratory, where the experiment was carried out.

- Larval Rearing:

The eggs were incubated in a net-cage inside a 500ℓ tank, under moderate aeration and slow running water system. The newly hatched larvae were transferred to nine plastic transparent aquaria with 50ℓ each, where rearing was conducted at a density of 12 larvae/ℓ.

Marine "chlorella" (*Nannochloropsis oculata*) was introduced in each aquaria at a concentration of 1.0×10^6 cells/mℓ, in stagnant water and with aeration at a rate of 50-100mℓ/min. In order to keep marine "chlorella" density, daily monitoring was done until the 5^{th} day, when the running water system started (at first with 1/2 turnover/day and with 1 turnover/day after the 7^{th} day).

Live foods organisms provided to larvae included rotifer (*Brachionus plicatilis*) and *Artemia* nauplii. Feeding in all aquaria was conducted as follow: rotifer from the begining to the end of the rearing period and *Artemia* nauplii from the 18^{th} day to the end of the experiment. Rotifer was introduced one day after hatching. Rotifer fed on marine "chlorella" was supplied daily in all aquaria until the 6^{th} day, when the specific treatments started. Three kinds of diets were tested: treatment 1 (T-1) - rotifer fed on marine "chlorella"; treatment 2 (T-2) - rotifer fed with marine "chlorella" and baker's yeast; and treatment 3 (T-3) - rotifer fed with baker's yeast and enriched by direct method (emulsion oil). The amount of food supplied was: rotifer given once a day, at first, and twice a day from the 10^{th} day, when the larvae were more developed and active, to maintain its density at 10 ind./mℓ. However, from the 18^{th} day to the 20^{th} day, due to a shortage in the rotifer culture, the amount was reduced to 5 ind./mℓ, and *Artemia* nauplii, enriched with fish oil, was introduced, once or twice a day, to maintain its density at 0.2 ind./mℓ.

The temperature was monitored everyday. Measures of pH were made from the 6^{th} day to the end of the experiment, every 2 days, and the bottom was cleaned, by syphoning, to remove detritus, once a day.

The total length (TL) and body depth (BD) were measure everyday during the experimental period. Two larvae of each aquaria were measured, in a total of six larvae per treatment. The growing curve was drawn for each treatment.

At the end of the experiment, 30 larvae per treatment were measured in TL and BD and the survival rate was obtained by counting all the alive larvae in each aquaria. The mortality was estimated in two periods: 1) from the 1^{st} to the 17^{th} day; and 2) from the 18^{th} to the 22^{nd} day (end of the experiment). During the 2^{nd} period mentioned, the number of dead larvae was counted everyday while, for the 1^{st} period, the number of dead larvae was estimated by the mortality during all the experimental period (100% - survival rate %), minus the mortality during the 2^{nd} period.

The experimental design was completely randomized with 3 replication to each treatments. The analysis of variance (ANOVA) was applied for the finals results of TL, BD and survival rate, in order to verify if there was any significative difference between treatments.

- Live Food Organisms Production:

The rotifer culture was conducted in nine tanks of 30ℓ at 25% of salinity, temperature between 25 and 28°C, with constant aeration. Rotifer was fed with three kinds of food: 1) only marine "chlorella"; 2) marine "chlorella" and baker's yeast; and 3) only baker's yeast. The harvest was total or partial, depending on rotifer concentration. The water was changed every 3 days or when the water quality was not good and many contaminants were found. The amount of baker's yeast given was 1.0g/1,000,000 rotifers once every 2 days for treatment 2 and the same amount everyday for treatment 3.

In case of the treatment 3, the rotifers fed only with baker's yeast, it were immersed in a solution of emulsion oil, 12 hours before to be provided to the larvae. The emulsion was prepared by mixing egg yolk (1.0g), destilled water (100mℓ) and fish liver oil (5.0g) in a homogenator for 2-3min. The utilized dosage was 1.0mℓ/1,500,000 rotifers.

The incubation of *Artemia* cysts (5.0g) was conducted in a 30ℓ tank at 35% of salinity, temperature between 25°C and 28°C with constant aeration. After 15 to 20 hours, aeration was stoped and the tank covered to collect the nauplii, that were enriched by fish oil (1.0mℓ/ℓ), 3 to 5 hours prior to be provided to larvae.

RESULTS AND DISCUSSION

Red seabream larvae utilized in the experiment hatched between 50 and 60 hours after fertilization, with the hatching rate estimated from 90% to 95%. Larvae started to be fed 27 hours after hatching, when 2/3 of the yolk sac was consumed and the development of the digestive tract was observed. According FUKUHARA (1984), the yolk sac is completelly consumed 3 days after hatching and the mouth opens after 2 days, when the larvae start feeding and a suitable amount of food should be available (table 1).

Newly hatched larvae measured 2.38mm in average and at the end of the experiment the results showed: 7.84mm ± 0.04 & 1.94mm ± 0.31 for treatment 1; 7.63mm ± 1.11 & 1.88mm ± 0.68 for treatment 2; and 7.08mm ± 0.58 & 1.74mm ± 0.17 for treatment 3, in TL and BD, respectivelly (table 1 & 2). Referring the utilized methodology (ANOVA), no significative difference was found at a level of 5% of probability by the F' test (F = 0.88 for TL and F = 0.38 for BD) (table 2). Nevertheless, as shown in figure 2 and 3, no difference was observed until the 17th day, but a steadly tendency can be seen in the growing curves, both for TL and BD. The fluctuation showed probably was due by the reduced number in each sampling. The results show in table 1 that a shortage of rotifer occurred in the treatment 3 at the 11th day, when 6 ind./mℓ was utilized as the concentration, immediatelly recovered in the next day. But after the 14th day until the 20th day, problems with rotifer in the same treatment occurred at the same level, while the concentration in the others two treatments was maintained in 10 ind./mℓ and only after the 18th day the concentration of rotifer in all treatments was reduced for the availability in the treatment 3 (5 ind./mℓ). Probably this fact could have affected the growth development in treatment 3 as shown in fig. 2 and 3, but it didn't affect the survival rate.

According KURUNUMA & FUKUSHO (1984), there are three critical periods observed in the rearing of red seabream larvae. The first comes about 4 days after hatching, the second between 12 and 17 days after hatching and the third comes 20 to 25 days after hatching. In the present experiment, two distinct phases were clarified. The first one in the 17th day of the rearing period, when mortality of larvae was calculated in 23.8%, 14.9% and 14.3%, and the second from the 18th to the 22nd day, with 8.3%, 15.5% and 15.4%, for treatment 1, 2 and 3, respectivelly. For the former phase the probable reason should have been the insufficiency of rotifer as a diet for larvae, showing that feeding only rotifer was not enough for the larvae requirement and another kind of live organism, i.e. copepods, must be considered as an intermediary diet during this phase. At the latter phase, the cause was probably the change on the kind of food supplied, when *Artemia* nauplii was introduced. Also was observed difference in size among larvae and probably some were not yet apt to feed on *Artemia* nauplii.

The survival rate at the end of the experiment was 67.8% ± 8.17, 69.6% ± 6.99 and 70.3% ± 6.95, for treatment 1, 2 and 3, respectivelly, once more confirming the already above described, that no significative difference was found. In the treatment 3, the survival rate was slightly higher. However, the larvae showed a slightly inferior growth if compared with the others two treatmets, but, their inferior growth could be due to a higher number of larvae in the aquaria, resulting in less developed larvae.

During the rearing period, the temperature ranged between 18.7°C and 21.7°C and the pH between 8.0 and 8.6 (fig. 1 and table 4). The pH varied slightly among aquaria, demonstrating that it was not a cause of the treatments.

At the end of the experiment, the total amount of food given to the larvae was calculated, with the aim to record the necessity of each item in the rearing of red seabream larvae (table 3). Considering this information, it was possible to estimate for a commercial scale, a production of aproximately 30.00 x 10^9 rotifers will be necessary to rear 1,000,000 larvae of red seabream for 22 days. The data show the importance of rotifers as a primary food for red seabream larvae, so they must be carefully cultured and handled to ensure the proper supply for larvae requirement.

So rotifer fed on baker's yeast enriched by emulsion oil, can be considered a good alternative for the rearing of red seabream larvae, when microalgae are not available in terms of quantity and quality, since the results reached in this experiment show, by the statistical analysis of variance, that no significative difference was found between treatments, for the survival and growth of larvae.

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- Larval Development of Red Seabream (*Pagrus major*)

Date	Period	Water Temp. (°C)	T-1		T-2		T-3		Observations
			T.L. (mm)	B.D. (mm)	T.L. (mm)	B.D. (mm)	T.L. (mm)	B.D. (mm)	
04/27	0	19.8	2.38	-	2.38	-	2.38	-	All larvae hatched (2pm). Hatching rate estimated between 90 to 95%. Larvae were transfered from incubator to nine aquaria at a density of 12 larvae/ℓ. Marine "chlorella" was introduced in each aquaria (1×10^6 cells/mℓ) with stagnant water and aeration at a rate of 50-100mℓ/ℓ.
04/28	19h	20.2	2.97	-	3.02	-	2.98	-	1/3 of yolk sac was absorbed.
	27h	21.4	-	-	-	-	-	-	2/3 of yolk sac was absorbed. Rotifer was introduced in the aquaria at a density of 10 ind./mℓ.
04/29	1d19h	21.2	3.40	-	3.37	-	3.37	-	Development of digestive tract. All yolk sac was absorbed.
04/30	2d19h	21.0	3.45	0.67	3.47	0.62	3.43	0.65	
05/01	3d19h	20.1	3.43	0.69	3.49	0.79	3.35	0.68	
05/02	5d 1h	19.4	3.64	0.81	3.68	0.87	3.74	0.83	Start running water system (1/2 turnover/day).
05/03	6d	18.7	4.00	0.70	3.98	0.73	3.98	0.77	Start specific treatments.
05/04	6d19h	19.2	4.12	0.75	4.05	0.77	4.28	0.83	Increase of running water to 1 turnover/day.

Continue

Conclusion

Date	Period	Water Temp. (°C)	T-1		T-2		T-3		Observations
			T.L. (mm)	B.D. (mm)	T.L. (mm)	B.D. (mm)	T.L. (mm)	B.D. (mm)	
05/05	7d19h	19.6	4.63	0.89	4.50	0.91	4.38	0.88	
05/06	8d20h	20.2	4.80	1.03	4.52	0.95	4.73	0.97	
05/07	9d20h	19.8	4.86	0.83	4.88	0.97	4.52	0.87	Rotifer start to be added twice a day. Development of air bladder.
05/08	10d19h	19.9	4.96	1.00	4.90	1.04	4.95	1.06	
05/09	11d19h	21.7	5.03	1.12	5.48	1.20	5.25	1.14	
05/10	12d19h	-	5.70	1.31	5.80	1.33	5.53	1.22	
05/11	13d19h	21.5	5.61	1.29	6.03	1.42	5.94	1.65	
05/12	14d19h	21.5	6.09	1.44	6.36	1.43	6.11	1.47	
05/14	16d19h	-	6.25	1.45	6.55	1.59	6.11	1.33	
05/15	18d 2h	21.2	6.45	1.54	6.93	1.74	5.97	1.31	Start feeding with *Artemia* (0.2 ind./mℓ). *Artemia* was enriched with fish oil (1mℓ/ℓ) during 3-5 hours. In the morning, larvae were fed with rotifer at 5 ind./mℓ for all treatments.
05/16	19d 2h	20.7	6.88	1.56	7.48	1.85	5.26	1.48	
05/17	20d 3h	20.7	7.48	1.84	7.27	1.88	6.47	1.59	
05/19	22d 3h	21.2	7.84	1.94	7.63	1.88	7.08	1.74	End of the experiment.

Tab. 2 - Total lenght (T.L.), body depth (B.D.) and survival rate (S.V.) in the end of the experiment and mortality (M) in two periods of rearing for each treatment.

	T-1	T-2	T-3	$F^*_{.95}$
T.L. (mm) (Mean ± S.D. n=30)	7.84 ± 0,04	7.63 ± 1.11	7.08 ± 0.58	0.88 ns
B.D. (mm) (Mean ± S.D. n=30)	1.94 ± 0,31	1.88 ± 0.68	1.74 ± 0.17	0.38 ns
M(%) (until the 17th day)**	23.8	14.9	14.3	
M(%) (18th - 22nd day)	8.3	15.5	15.4	
S.V. (%) (Mean ± S.D.)	67.8 ± 8.17	69.6 ± 6.99	70.3 ± 6.95	0.09 ns

*ANOVA

**Value estimated by the mortality during the experimental period minus the mortality from the 18th to the 22nd day.

Tab. 3 - Total amount of items consumed during the experimental period for each treatment.

	T-1	T-2	T-3
Marine "chlorella"	1,400ℓ	1,000ℓ	-
Baker's yeast	-	96.45g	50.04g
Emulsion oil	-	-	21mℓ
Rotifer	40.6 x 10^6 ind	38.975 x 10^6 ind	37.975 x 10^6 ind
Artemia	5.0 x 10^4 ind	5.0 x 10^4 ind	5.0 x 10^4 ind
Fish oil for *Artemia*	3mℓ	3mℓ	3mℓ
Total nº of larvae produced	1,221 larvae	1,253 larvae	1,266 larvae

Tab. 4 - Data on pH in each aquarium.

Date	Time	T-1			T-2			T-3		
		1	2	3	1	2	3	1	2	3
05/03	4pm	8.15	8.35	8.40	8.55	8.65	8.65	8.60	8.55	8.45
05/05	4pm	8.10	8.20	8.30	8.30	8.20	8.35	8.40	8.35	8.30
05/07	10am	8.00	8.10	8.20	8.30	8.35	8.40	8.40	8.35	8.35
05/09	11am	8.10	8.30	8.40	8.50	8.55	8.65	8.60	8.55	8.45
05/11	9am	8.00	8.05	8.10	8.15	8.05	8.30	8.25	8.20	8.15
05/15	2pm	8.00	8.15	8.10	8.20	8.20	8.30	8.20	8.25	8.15
05/17	9am	8.10	8.20	8.30	8.30	8.35	8.40	8.35	8.35	8.35

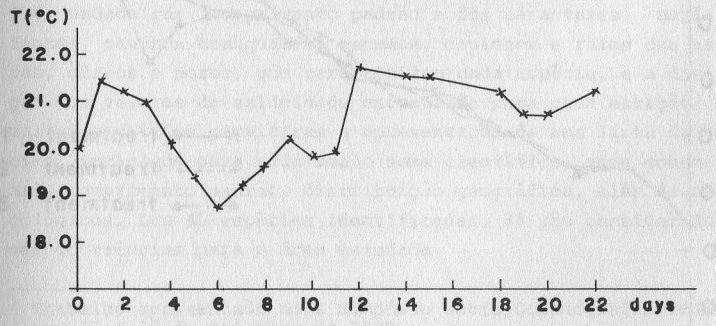

Fig. 1: Fluctuation of temperature during the experimental period

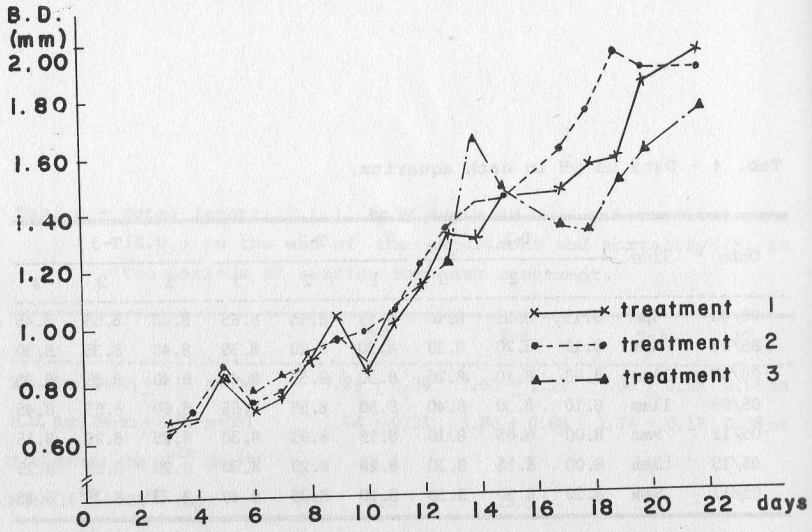

Fig. 2: Larval growing curve in body depth (B.D.) for each treatment

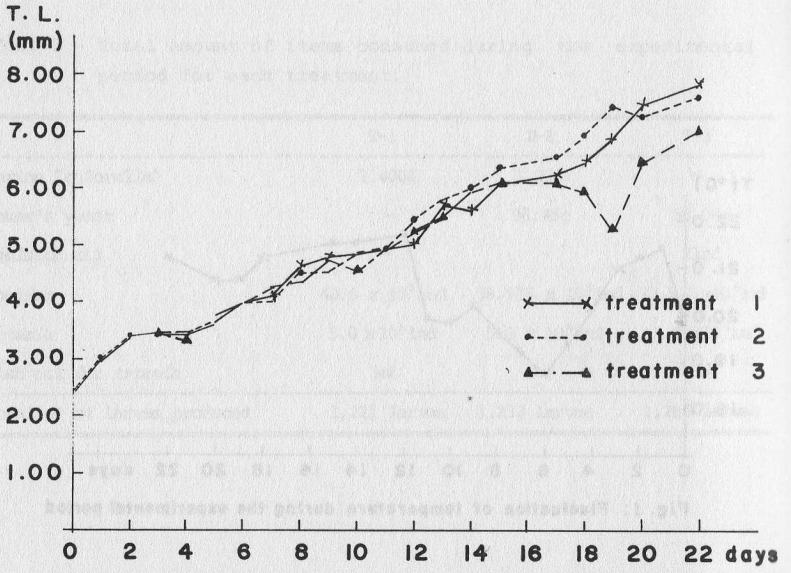

Fig.3: Larval growing curve in total lenght (T.L.) for each treatment