Effect of a rotifer and artemia on survival and growth performance of gilthead seabream *Sparus aurata* larvae.

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ABSTRACT

An experiment was carried out in order to study the effect of a rotifer *Brachionus plicatilis* and encapsulated *Artemia nauplii as a* live food on survival rate and growth performance for gilthead sea bream *Sparus aurata* larvae. Gilthead sea bream larvae (20 days old) with body length and weight of (7.3 $\pm$ 0.20 mm) (5.4 $\pm$ 0.10 mg) respectively, were stocked in eight white fiberglass tanks (each of 1 m^3 volume) at a density of 1,200-larvae/ tank. Four treatments were tested as follows, 1) low rotifers and low artemia (LRLA); 2) high rotifers and low artemia (HRLA); 3) low rotifers and high artemia (LRHA); and 4) high rotifers and high artemia (HRHA) for 24 days (5, 25 rotifers and 50, 250 artemia /ml, during the periods 1st, 8th, 9th, 16th, 17th and 24th days of the feeding experiment).

The results showed that the survival rate of *S.aurata* larvae significantly ($P \leq 0.05$) improved with the application of the experimental program by feeding a high mixture of rotifers and artemia. Feeding of higher levels of rotifers and artemia increased the survival rate of *S. aurata to* 48.96%, however, the lower levels of both resulted in only 12.17 % survival rate. Other intermediate treatments of HRLA or LRHA resulted in better improvements in the survival rates (19.08 and 32.21% respectively) of *S. aurata* larvae. The results clearly showed the significant role of higher levels of encapsulated *A. nauplii* as a live food for saving the life of *S. aurata* larvae as compared with rotifers.

Values of growth performance of *S. aurata* larvae (gain in length and weight; average daily gain in length and weight; and specific growth rate, (SGR %) significantly ($P \leq 0.05$) increased with increasing the levels of live food from both rotifers and artemia. The results clearly showed the superiority of the higher levels of live artemia in enhancing growth performance of *S. aurata* larvae than rotifers. Finally, a suitable live food program to improve the survival rates of *S. aurata* larvae by feeding high levels of rotifers and artemia could be recommended.

Keywords: Rotifers, Artemia, Sea bream, Survival, Growth performance

INTRODUCTION

Seed production and larval rearing stay very important for all growing operations and considered as the main limiting factors for development of finfish Mari culture (Dhert *et al*., 1998). Starvation is a major problem for larvae with small reserves of endogenous energy. It is not easy to quantify the nutritional requirements of larval fish. However, it is believed that the optimal formulations for first-feeding larvae should simulate the yolk composition and to some extent reflect the nutrient requirements and metabolic capacities of pre-feeding fish (Heming and Buddington, 1988). The variability of the nutritional value of live foods for marine larval fish is well documented (Watanabe *et al*., 1980; Bottino *et al*., 1980; Kuhlmann *et al*., 1981; and Leger, 1986). The importance of small live preys (organisms) especially rotifers

and artemia for marine fish hatcheries success has been stressed. Rotifers are valuable live food for larval fish and crustacean culture. Several characteristics of rotifers, including their nutritional quality, body size and relatively slow motility have contributed to their usefulness as good prey for active larvae (Reitan, *et al*., 1997). The rotifer *Brachionus plicatilis* has been most widely used as essential food source for raising marine environment (Lubzens, 1987; Dhert *et al*., 1994). Zaki *et al*. (2003) showed that the concentration of 16 rotifers per ml of water was optimum for feeding gilthead sea bream *S. aurata* larvae. This concentration in enough to ensure the formation of functional swim-bladder inflation, which improve, the abilities of larval fish for swimming, hunting, feeding, growing, and preventing deformities in fish fry. The rotifer *Brachionus plicatilis* (S-type Hawaiian strain) was cultured with various combinations of baker's yeast and *Nannochloropsis oculata* to feed different fish larvae (Clyde *et al* .,2003).

Artemia nauplius is essential for larval culture of fish hatcheries (Van stappen and Sorgeloos, 1993). Due to its high nutritional value, suitable size, mobility, biochemical composition, *A. nauplii* has high interest in larval fish culture .In addition, the possibility for improving its nutritional manipulation through enrichment increased the importance of artemia as a live food (Sorgeloos, 1994). There were three main critical phases in intensive rearing of larval sea bream *S. aurata* :a) the end of the larval stage (day 3-4);b) the endoexotroph stage (day 8-12);c) the larval stage (day 25-35).More than 99 % of the fry were lost during these phases (Dhert *et al*., 1998)and mean survival was seldom greater than 10 % .This low survival was often made worth by cannibalism and abnormalities of marketable fry during nursery operations (Lagos,1989). A new culturing technique using rotifers and artemia as a live foods were developed in Thailand for larval fish production with about 40 % survival rate during the hatchery and nursery phases (Maneewong *et al*., 1986 a,b).

The aim of the present work was to improve the survival rate and growth performance of gilthead sea bream *S.aurata* in a commercial hatchery through application of different live food regimes.

MATERIALS AND METHODS

This study was undertaken in the Governmental Marine Finfish Hatchery, Km21, Alexandria, at the General Authority for Fish Resources Development, (GAFRD), Ministry of Agriculture, in order to study the effect of the rotifer *Brachionus plicatilis* and the encapsulated *A. nauplii* as a live food on survival rate and growth performance for gilthead sea bream *S. aurata* larvae. This experiment was performed in eight white circular fiberglass tanks, each of 1m3 water volume in black greenhouse.

The Microalga *Nannochloropsis oculata* and the rotifer *Brachionus rotundiformis* were cultured in a greenhouse as a semi-continuous system (harvesting 20-30% daily), using fiberglass tanks of 250 and 500-liter capacity, respectively. A mixture of agricultural-grade fertilizer was the culture medium used for microalgae (1 liter of freshwater solution, containing 150 g ammonium sulfate, 25 g super phosphate, and 7.5 g urea, for 1000 liters of seawater added to the culture). Temperature range was 22-26°C. One of the rotifer cultures received 100 x 103 microalgae cells per individual/day, while the other received 50 x 103 microalgae cells per individual/day and 0.5 µg of baker's yeast per individual/day (rotifers were fed three different diets which represented the three treatments, with three replicates: treatment A, rotifers fed only microalgae; treatment B, rotifers fed microalgae and

baker's yeast (1:1); treatment C, rotifers fed microalgae and baker's yeast (1:1) enriched with commercial emulsion, according to the manual of Oceanic Institute (1995). Rotifer enrichment was done with a commercial emulsion (Selco® from INVE Aquaculture NV, Basrode, Belgium), in 40-liter cylindric-conical tanks at a density of 400 x 103 individuals/liter over 15h with 0.2 g/l of the product, following manufacturer instructions. Artemia cysts collected from salt works at El-Max for salines Co. Alexandria, and incubated for 48 hrs at 25 ppt salinity to get good hatching ratio. The average diameter of artemia cysts was 254.5 μm and the average length of its nauplii was 487.8 μm.

Two types of live foods the rotifer *B. plicatilis* and the encapsulated *A. nauplii* were tested in four treatments, 1) low level of rotifers and low artemia (LRLA) (control); 2) high level of rotifers and low level of artemia (HRLA); 3) low level of rotifers and high level of artemia (LRHA); and 4) high level of rotifers and high level of artemia (HRHA), (Low and high levels were 5, 25 and 50, 250 prey/ml rotifer and artemia respectively during the periods 1^{st}, 8^{th}, 9^{th},16^{th}, 17^{th} and 24^{th} days of the feeding experiment).

Approximately 30h after hatching, larvae of the Gilthead sea bream *S. aurata* were placed in one fiberglass tank ($1m^3$ liter). The tank was equipped with an airlifting system. Daylight fluorescent light provided a light intensity of 1000 lux at the water surface, with a photoperiod of 12hr light: 12hr dark (12L:12D). On the 20^{th} day after hatching, all surviving larvae were counted and 50 larvae were measured, using a dissecting microscope equipped with an mocular micrometer (standard average length and average body weight 7.3$\pm$ 0.20mm and of 5.4 $\pm$ 0.10 mg. Eight fiberglass tanks used of rearing larvae were stocked as a density of 1200 larvae /tank on 15 May 2007.

Water exchange was approximately 15% per day. Temperature was monitored at 8:00 h daily, while salinity, ammonia, pH and dissolved oxygen were measured every six days, in tanks of each treatment. Temperature (21.3-26.°C), non-ionized ammonia (0.07-0.10 mg/l), dissolved oxygen (4.0-5.5 mg/l), salinity (35 g/l) and pH (8.0) did not differ significantly among treatments.

Fish larvae used in the present experiment were produced from tank-matured brood stocks (4 years old and average weight of 750-1000g). Brood stocks were previously spawned artificially at a water temperature range from 16-18C°, using LHRH-hormone pellets.

Seawater (35 ppt) was pumped via a sand filter and passed through clothes' filter (200 micron) before being entered to the tanks. Water exchange rate was 40, 50, and 60 %daily during the periods 1^{st}, 8^{th}, 9^{th}, 16^{th}, 17^{th} and 24^{th} days of the experiment, respectively. Each tank was equipped with standpipe fitted with Nylon screen (100 micron) to prevent the rotifers from escaping. Photoperiod was maintained at Light intensity during the experimental period of 200 lux, by installing 100-watt lamp over water surface, besides the fluorescent lamps hanged in the greenhouse. Tanks were siphoned once every day. A floating oil trap was used to remove the oil film on the water surface.

Nannochloropsis oculata was added to rotifers rearing tank at a density of 200.000 cells/ml during the experimental period. The rotifer *Brachionus plicatilis* was grown on the micro alga *Nannochloropsis oculata* while the rotifers were washed for 10 minutes before adding to larval rearing tanks.

At the end of the experiment, samples of 50 fish larvae were weighed and the average total length and weight in each treatment were measured to calculate the final weight, average daily length and gain, and specific growth rate (SGR % in length and

weight). Larval survival rate was calculated after 24 days through counting the total number of the produced fish. Measurements mentioned were calculated according to the following formula:

Fish survival rate (%) = 100 (FN / IN)

Where: FN: number of fish at the end of the experiment

IL: number of fish at the beginning of the experiment, (Akatsu *et al.*, 1983)

Average daily length (ADL) = (FL - IL) / T

Where: FL: mean length at the end of the experiment

IL: mean length at the beginning of the experiment

T: time in days

Average daily gain (ADG) = (FW - IW) / T

Where: FW: mean weight at the end of the experiment

IW: mean weight at the beginning of the experiment

T: time in days

Specific growth rate in length (SGR%) = 100 (ln FL - ln IL) / T

Where: FL: mean length at the end of the experiment

IL: mean length at the beginning of the experiment

T: time in days (Jauncey and Ross, 1982)

Specific growth rate in weight (SGR%) = 100 (ln FW - ln IW) / T

Where: FW mean weight at the end of the experiment

IW means weight at the beginning of the experiment

T: time in days (Jauncey and Ross, 1982)

Condition factor =100 *final weight /(total length)^3

Water temperature and dissolved oxygen were measured daily, using oxygen meter (SPER Scientific), while pH values were recorded twice a week, using an advanced pH meter (840035 SPER Scientific). Water salinity was measured, using temperature compensated refractmeter.

Statistical analysis was performed using SPSS (Version 10 program) and treatments were evaluated at the 0.05 probability. Analyses of variance, one – way ANOVA was used to evaluate the effect of live food on survival rate, total body length and weight, average daily length and weight gain and Specific growth rate in length and weight. The differences within treatments using LSD were used at 0.05 probability (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Throughout the culture period, the averages of water quality criteria for larval rearing tanks was as follows: salinity 35 ppt; temperature 19.6C°; dissolved oxygen DO_2 6.9 ppm, and pH 8.02. Similar parameters have been reported by Zaki *et al.*, (2003) and Nour *et al*., (2003).

Results in Table 1 show the effect of four different treatments of live food of a mixture of rotifers and artemia (LRLA, HRLA, LRHA and HRHA) on survival and growth performance of *S. aurata* larva. Survival rate % of *S. aurata* larvae significantly ($P \leq 0.05$) increased from 12.17% with LRLA to 48.96 %with HRHA. Higher concentrations of each of rotifers or arteria resulted in a better survival rate of *S. aurata* larva as compared with the control group (LRLA). However, the significant role of artemia over rotifers was clearly observed in the differences between the 2nd (19.08) and 3rd (32.21%) treatments respectively.

Growth performance of *S. aurata* larvae fed different combinations of live food (LRLA, HRLA, LRHA and HRHA) showed a significant ($P \leq 0.05$) increase in larval length and weight, length and weight gain, average daily length and weight and specific growth rate (SGR %) in length and weight (Table 1). Growth in length showed the superiority of artemia (treatment HRLA) as a live food for *S. aurata* larvae over rotifers (treatment LRHA). While

higher concentrations of both achieved a higher growth of length (treatment HRHA).On the other hand, growth in weight showed a similar trend in growth in length. Dhert *et al*. (1998) mentioned that there are three main critical and sensitive phases of larval age as follows: 1-the end of pre-larval stage (day 3-4), 2- the endo-exotroph stage (days 8-12), and 3-the larval stage (days 25-35). The results showed that when *S. aurata* larvae reach 5.0-5.2 mm (15-20days after hatching), they start feeding on newly hatched rotifers and Artemia, while the most important factors affecting survival and growth of larvae in the hatcheries is the quantity and the quality of food during the most critical periods before weaning of larval fish until the complete formation of swim-bladder. One of the most important food items during this period is the rotifer *Brachionus plicatilis* (Dhert, 1996). This kind of natural food is very suitable for feeding many species of marine fishes, such as the sea bream *Sparus aurata*, sea bass *Dicentrarachus labrax*, halibut, turbot, sole, Red Sea bream, flat fish, clown fish, Japanese blue crab and the prawn *Penaeus japonicus* (Hoff and Snell, 1993). Crespo *et al*. (2001) mentioned that the nutritional factors, rather than infectious agents, are responsible for the high mortality encountered in the cultured dentex larvae. Rotifers has many advantages: 1- the possibility of rearing larvae at very high densities up to 2000 larvae / ml (Reitan *et al*., 1994); 2- tolerate a wide range of culture conditions; 3- have high reproduction rate; 4- of planktonic nature (Dhert, 1996), 5- with many sizes, to it suitable for many species and ages of fish and shrimp larvae, and 6-can be cultured on cheap formulated feeds. The results of Zaki *et al*. (2003) clearly show the importance of larvae survival and growth performance using the natural food organisms like rotifers). They concluded that 16 rotifers /ml is the optimum density required for gilthead sea bream larvae during the 1st to 21st days of their life. That concentration of rotifers significantly ($P<0.05$) increased swim-bladder inflation, growth performance, survival rate and decreased malformations of the larvae. The authors found that about 1067 rotifers were required for each 1 mm increase in larval length during the period from the 2nd to 21st days of its life in an industrial commercial hatchery in Egypt.

The survival and growth rate of *S. aurata* larvae were greatly improved when high levels of rotifers were added together with artemia (treatment 4). The positive effect of artemia to *S. aurata* larvae during this stage of life appear to enhance appetite of the larvae. Ganzon-Naret (1994) reported that delaying feeding of artemia nauplii until day 15 resulted in slower growth rate of sea bass fed *Artemia nauplii* starting on day10. Further studies of the mechanisms and interactions between rotifer-larval and artemia larval interactions at various steps of the larval feeding process should be given high priority in future research. The present results clearly show that higher levels of rotifers and or artemia significantly ($P<0.05$) decreased the condition factor (K) values from 0.71 to 0.57 respectively (Table 1).

Table 1: Effect of rotifers and artemia as a live food on survival rate and growth Performance of gilthead Sea bream, *Sparus aurata* larvae.

Items	Treatments			
	LRLA	HRLA	LRHA	HRHA
a) Survival rate (%)				
Survival rate (%)	12.17^{d}	19.08^{c}	32.21^{b}	48.96^{a}
b) Growth in length				
Initial length (mm/pce)	7.30^{a}	7.30^{a}	7.30^{a}	7.30^{a}
Final length (mm/pce)	11.50^{d}	12.80^{c}	14.30^{b}	15.65^{a}
Length gain (mm/pce)	4.20^{d}	5.50^{c}	7.05^{b}	8.35^{a}
ADL (mm/pce/day)	0.18^{d}	0.23^{c}	0.29^{b}	0.35^{a}
SGR in length (%/day)	1.89^{c}	2.34^{bc}	2.80^{ab}	3.27^{a}
c) Growth in weight				
Initial weight (mg/pce)	5.40^{a}	5.40^{a}	5.40^{a}	5.40^{a}
Final weight (mg/pce)	10.85^{d}	13.75^{c}	18.15^{b}	21.95^{a}
Weight gain (mg/pce)	5.45^{d}	8.35^{c}	12.75^{b}	16.55^{a}
ADG (mg/pce/day)	0.23^{d}	0.35^{c}	0.53^{b}	0.69^{a}
SGR in weight (%/day)	2.91^{d}	3.89^{c}	5.05^{b}	5.84^{a}
d) Condition factor (%)3				
Condition factor(%)	0.71	0.66	0.62	0.57

LRLA =low rotifers and low artemia; HRLA = high rotifers and low artemia; LRHA = low rotifers and high artemia; HRHA = high rotifers and high artemia.
Low rotifers = 5 pcs /ml; high rotifers = 25 pcs /ml
Low artemia = 50: high artemia =200 pcs /ml
Means in the same row with different superscripts are significantly different ($p \leq 0.05$)

ACKNOWLEDGEMENT

The authors' wishes to express our thanks to the stuff members in the Governmental Marine Finfish Hatchery, Km21, Alexandria of the General Authority for Fish Resources Development, (GAFRD), Ministry of Agriculture, Alexandria, Egypt for the facilities offered throughout this work.

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ARABIC SUMMARY

تأثير استخدام الروتيفر والارتيميا على معدلات البقاء وكفاءة النمو ليرقات اسماك الدنيس

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نفذت هذه التجربة لدراسة تأثير الغذاء الطبيعي من الروتيفر والارتيميا على معدل الإعاشة وكفاءة النمو ليرقات اسماك الدنيس لمدة ٢٤ يوم ، حيث تم استخدام يرقات الدنيس بعمر (٢٠ يوما) و طول ووزن جسم (٧,٣ $\pm$ ٠,٢٠ ملم)،(٥,٤ $\pm$ ٠,١٠ ملج)على التوالي بعد استخدام ثمانية تانكات فيبر جلاس سعه كل منها واحد متر مكعب وبكثافة ١٢٠٠ يرقة / تانك. كما تم استخدام اربع معاملات من خليط التغذية من الغذاء الطبيعي الروتيفر بمعدل ٥ و٢٥ فردا / مل والارتيميا بمعدل ٥٠ و٢٥٠ فردا /مل (للمستوى المنخفض والعالي على التوالي) وكانت التغذية على النحو التالي :

١- مستوى منخفض من الروتيفر و الارتيميا .
٢- مستوى عالي من الروتيفر ومنخفض من الارتيميا .
٣- مستوى منخفض من الروتيفر وعالي من الارتيميا
٤- مستوى عالي من الروتيفر والارتيميا

قد أظهرت النتائج أن هناك اختلافات ذات دلاله إحصائية ($P>0.05$) لمعدل الاعاشه حيث تحسنت مع استخدام المستويات العالية من الروتيفر والارتيميا . في حين وجد ان كفاءة النمو ليرقات اسماك الدنيس (الطول والوزن ؛ معدل النموالليومي في الطول والوزن ، ومعدل النمو النوعي) تحسنت بشكل كبير($P>0.05$) مع زيادة مستويات التغذية على الغذاء الطبيعي.

وتوصي الدراسة باهمية استخدام الغذاء الطبيعي وبمستويات عالية ليرقات اسماك الدنيس حيث يجعلها متاحة امام اليرقات في هذه المرحلة من العمر.