GROWTH PERFORMANCE AND SURVIVAL OF GILTHEAD SEABREAM *SPARUS AURATA* LARVAE FED ROTIFER AND ARTEMIA

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SUMMARY

The 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 + 0.20 mm) (5.4 + 0.10 mg) respectively, were stocked in twelve white fiberglass tanks (each of 400 Liter volumes) at a density of 1200-larvae/tank. 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 (10, 30 rotifers and 100, 200 artemia /ml, during the periods 1st, 8th, 9th , 16th, 17th and 24th days of the feeding experiment). 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. Values of growth performance of *S. aurata* larvae (gain in length and weight; average daily (gain in length and weight; and specific growth rate) were significantly 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

Even though the species *Sparus aurata* (L., 1758) is widely known in the Mediterranean region as a valuable fishery product with very good market price, the knowledge of it's special characteristics is incomplete while most information from experiments (mainly rearing data) are reserved and patented from big hatcheries and farms (Conides, 1992).Marine fish cultivation within the Egyptian mariculture industry has shown a rapid improvement for the last 5 years. The number of hatcheries dealing with larval production has increased up to 19 and the number of enterprises dealing with cultivation up to 243. 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*., 2004) showed that the concentration of 16 rotifers per ml of water was optimum for feeding gilthead sea bream *S. aurata* larvae. This concentration is 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).

An overlapping co-feeding period during which live food is gradually replaced by increasing quantities of formulated feed has been shown to improve growth and survival of marine fish larvae compared to the use of live food only (Curnow *et al.*, 2006 a, b and Eryalcin, 2018) determined the effects of several commercial rotifer feeds and enrichments on growth, biochemical and fatty acid composition of L-type rotifer and Artemia franciscana nauplii. Artemia *franciscana nauplii* enriched Red Pepper showed highest HUFA accumulation. 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 Haraz Marine Finfish Hatchery, Elkantra-Ismailia Governorate, 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 and stresstest for gilthead sea bream *S. aurata* larvae.

Experimental unit:

This experiment was performed in eight white circular fiberglass tanks, each of $1m^3$ water volume in black greenhouse. Twelve fiber glass tanks were used of rearing larvae on 15 January 2017. Seawater salinty (40 ppt) was pumped via a sand filter and passed through clothes, filter (200 micron) before being entered to the tanks. Water exchange rate was 15 % daily during the periods of experment. 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. The tank was equipped with an airlifting system. Tanks were siphoned once every day.

Experimental fish:

Fish larvae used in the present experiment were produced from tank-matured brood stocks (4 years old and average weight 1000g). Brood stocks artificially at a water temperature range of about 19 °C,. Approximately 30h after hatching, larvae of the Gilthead sea bream *S. aurata* were placed in one fiberglass tank. Larvae were stocked as a density of 1200 larvae /tank, Fish larvae were homogenous in body weights and apparently healthy. Fish were acclimated to farm conditions for 2 weeks' prior before the experiment study.

Environmental condition:

Daylight fluorescent light provided a light intensity of 1000 lux at the water surface, with a photoperiod of 16 H light: 8 H dark (16: 8 D). Water exchange was approximately 15% per day.

Experimental food:

The Microalga *Nannochloropsis oculata* and the rotifer *Brachionus rotundi formis* were cultured in a greenhouse as a semi-continuous system (harvesting 20% 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 was 19 ºC. One of the rotifer cultures received 100 x 10^3 microalgae cells per individual/day, while the other received 50 x 10^3 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 10^3 individuals/liter over 15H with 0.2 g/l of the product, following manufacturer instructions. Artemia cysts were 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,

Gilthead sea bream larvae:

Gilthead sea bream larvae were stocked in twelve fiberglass tanks (each of 400 L volume) at a density of 1,200 -larvae/ tank. Four treatments (with three fiberglass tanks) were tested as follows:

Low rotifers (10) and low artemia (100) (LRLA) (10,100); High rotifers (30) and low artemia (100) (HRLA) (30,100) ; Low rotifers (10) and high artemia (200) (LRHA) (10,200); and high rotifers (30) and high artemia (200) (HRHA) (30,200) for 24 days (10 , 30) rotifers and (100 , 200) artemia /ml), during the periods of the feeding experiment.

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.

Experimental methodology:

On the 20^{th} day after hatching, all surviving larvae were counted and 50 larvae were measured, using a dissecting microscope equipped with a mocular micrometer (standard average length and average body weight 7.3+ 0.20 mm and of 5.4 + 0.10 mg.

Fish were weighed every week and the average of 4 weeks was determined by UWE MJW-300 balance to the nearest 0.01g .The amount of diet was readjusted according to the new weight of fish.

Measurements:

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.

Growth performance parameters:

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

IN: 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

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

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

Water quality parameters:

Water exchange was approximately 15% per day. Temperature was monitored at 8:00 h daily, Water temperature and dissolved oxygen were measured by Metteler Toledo, model 128.s/No1242 respectively, Other water quality including pH and ammonia were measured every two days by pH meter (Orion model 720 A, s/no 13062) and ammonia meter by Hanna ammonia meter. The averages of water quality parameters are presented in Table (1). Water salinity was measured, using temperature compensated refractmeter.

Stress tests:

Fish from each rearing tank were removed carefully and directly transferred from salt water to fresh water (0.5-1 ppt). Osmotic shocks lasted for 1 hour. Moreover, the larvae were also suddenly exposed to thermal stress (15 °C for 1 hour). To regulate the temperature during the thermal stress, the tanks were equipped with ice and the water temperature was maintained at 15 °C. After stress period, fish larvae were sampled from each treatment for biochemical analysis and frozen at -80 °C.

Tissue extract and biochemical analysis:

Whole frozen larvae was defrosted, weighted and put into the mortar and added some liquid Nitrogen, then pressed with pestle samples. The samples were homogenated with electronic homogenizer at 4500×g, for 1.5 min and diluted with ratio of 1:9 deionized distilled water. Tissue suspensions were centrifuged at 10000×g for 15 min. at 0°C and frozen at -80 °C. Cortisol content was determined with Radio immune assay (RIA). The quantitative determination of glucose was carried out using commercially available diagnostic Experimental Protocol kits, Pars Azmoon, Iran (1 500 0178), at 546 nm and 37 °C by the glucose oxidase method according to Trinder (1969). The limit of detection (LOD) of the procedure was 5 mg/dl. Total protein was estimated following the method of Lowry *et al., (*1951).

Statistical analysis:

Statistical analysis was performed using SAS (1996). Analyses of variance, one-way ANOVA was used .Different between means were compared by Duncan's new multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Water quality:

Throughout the culture period, the averages of water quality criteria for larval rearing tanks (Table 1) were as follows: salinity 40 ppt; temperature 19 ºC; dissolved oxygen $DO_2$6.9 ppm, and pH 7. 2. Similar parameters have been reported by Zaki *et a*l. (2004) and Nour *et al*. (2004).

Table (1): Effect of rotifers and artemia as a live food of gilthead Sea bream, *Sparus aurata* larvae on water quality criteria.

Parameters	
Salinity	40 ppt
Temperature	19 ºC
Dissolving Oxygen	6.9 ppm
PH	7.2

Survival rate:

The group of larvae fed on (HRHA) had a significantly (P<0.05) highest (56.96%) survival rate than the rest of experimental groups (Table 2). The lowest survival rate (22.17%) was obtained in group of larvae fed on (LRLA). The survival of *S. aurata* larvae were greatly improved when high levels of rotifers were added together with artemia (Treatment 4).

Growth performance:

Growth performance of *S. aurata* larvae fed different combinations of live food (LRLA, HRLA, LRHA and HRHA) is presented in Table (2). Growth in length artemia (treatment HRLA) as a live food for *S. aurata* larvae over rotifers (treatment LRHA). 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 6.3 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 and Crespo *et al*., 2001). The present study reported 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 lowest condition factor (K) (0.36) was obtained on group of larvae fed on (HRHA). However, the highest condition factor (1.11) was obtained on group of larvae fed on (LRLA). The present results clearly show that higher levels of rotifers and or artemia significantly (P<0.05) decreased the condition factor (K) values from 0.36 to 1.11, respectively (Table 2). These values are within the international values for the good healthy fishes (Ogle, 2013). The results of Zaki *et al*. (2004) clearly showed the importance of larvae survival and growth performance using the natural food organisms like rotifers. They added that 16 rotifers /ml is the optimum density required for gilthead sea bream larvae during the 1st to 21st days of their life. The concentration of rotifers significantly (P<0.05) increased swim-bladder inflation, growth performance, survival rate and decreased malformations of the larvae.

Table (2): Effect of rotifers and artemia as a live food on survival rate and growth performance of gilthead Sea bream, *Sparus aurata* larvae.

Item	Treatment			
	LRLA	HRLA	LRHA	HRHA
a) Survival rate (%)	22.17^{d}	35.08^{c}	42.21^{b}	56.96^{a}
b) Growth in length				
Initial length (mm/pce)	6.30^{a}	6.30^{a}	6.30^{a}	6.30^{a}
Final length (mm/pce)	10.50^{d}	12.80^{c}	15.40^{b}	18.65^{a}
Length gain (mm/pce)	4.20^{d}	6.50^{c}	9.10^{b}	12.35^{a}
ADL (mm/pce/day)	0.18^{d}	0.27^{c}	0.38^{b}	0.51^{a}
SGR in length (%/day)	2.13^{c}	2.95^{bc}	3.42 ^{ab}	4.5^{0a}
c) Growth in weight				
Initial weight (mg/pce)	5.20^{a}	5.20^{a}	5.20a	5.20^{a}
Final weight (mg/pce)	12.85^{d}	15.75^{c}	19.15^{b}	23.95^{a}
Weight gain (mg/pce)	7.65^{d}	10.55^{b}	13.95^{b}	18.75^{a}
ADG (mg/pce/day)	0.32^{d}	0.44^{c}	0.58^{b}	0.78^{a}
SGR in weight (%/day)	2.91^{d}	3.76^{c}	5.42^{b}	6.36^{a}
d) Condition factor (%)				
Condition factor (%)	1.11	0.75	0.52	0.36

Means in the same row with different superscripts significantly different (P U< U 0.05)

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 = 10 pcs /ml; high rotifers = 30 pcs /ml Low artemia = 100: high artemia =200 pcs /ml and 6-can be cultured on cheap formulated feeds.

The positive effect of artemia to *S. aurata* larvae during this stage of life appears 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. He showed that when *S. aurata* larvae reach 6.0-6.3 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 japonicas*, also the nutritional factors, rather than infectious agents, are responsible for the high mortality encountered in the cultured density larvae (Hoff and Snell, 1993 and Crespo *et al*., 2001). 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.

Thermal and salinity shock:

The group of fish on HRHA had as significantly (P<0.05) highest survival rate than the rest of experimental group (Table 3). This is in agreement with Gapasin *et al*. (1998). Also, cotrisol contant was significantly highest in group of larvae fed on HRHA compared with the rest of experimental groups. In some fishes, cortisol is the principal corticosteroid (Jeney *et al*., 1992). Elevated cortisol levels cause gluconeogenesis and glucogenolysis in the liver (Mazeaud *et al*. 1977). The resulting hyperglycaemia helps to satisfy the increased energy demand during stress, allowing the organism to react to stressors (Grono, 1974). According to the results of this study, however, there was no significant difference between the groups in cortisol level of yellow fin seabream under stress conditions (P> 0.05).Total protein is measurable humoral

component of the non-specific defense mechanism (Jeney *et al.*, 1997). The total protein was significantly (P<0.05) highest in the fish fed HRHA than the other treatments after salinity stress but Very low levels of LALA group.

Table (3): Survival rate of seabream larvae after thermal and salinity shock.

Items	Treatments			
	LRLA	HRLA	LRHA	HRHA
Survival rate (%)	35.17[d]	40.08[c]	50..21[b]	60.96[a]
Cotrisol nm	1.00[a]	1.60[a]	2.40[a]	2.60[a]
Total protein ng	77.50[d]	82.80[c]	86.40[b]	122.65[a]
Glucose mg/dl	13.20[d]	13.50[c]	11.10[b]	12.35[a]

LRLA =low rotifers and low artemia; HRLA = high rotifers and low artemia; LRHA = low rotifers and high artemia; HRHA = high rotifers and high artemia.
[a, b, c and d] superscripts at the same row are significant (P<0.05)

Serum total protein in fish significance in relation to infectious disease, kidney damage, nutritional imbalance, stressful condition (Wedemeyer, 1981), and the principal corticosteroid (Jeney *et al*., 1992). Elevated cortisol levels cause gluconeogenesis and glucogenolysis in the liver (Mazeaud*et al*., 1977). The resulting hyperglycaemia helps to satisfy the increased energy demand during stress, allowing the orgaism to react to stressors (Gronow, 1974). According to the results of this study, however, there was no significant difference between the groups in cortisol level of yellow fin seabream under stress conditions (P < 0.05).

CONCLUSION

The present results clearly show that higher levels of rotifers and or artemia HRHA significantly (P<0.05) had highest survival rat, growth performance and lowest condition factor under this experimental condition.

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

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أجريت هذه التجربه لدراسة تأثير الغذاء الطبيعى من الروتبفروالارتيميا على معدل الاعاشه وكفاءة النمو ليرقات اسماك الدنيس لمدة 24 يوم فى مفرخ حراز البحرى على قناة السويس بالأسماعليه

حيث تم استخدام يرقات الدنيس بعمر (20 يوم) طول ووزن (7.3± 0.20ملم),(5.4±0.10ملج)على التوالى حيث تم استخدام 12تانك فيبرجلاس سعه كل واحد 400 لتر بكثافة 1200 يرقه لكل تانك ،كما تم اسخدام اربع معاملات من خليط التغذيه من الغذاء الطبيعى الروتيفر بمعدل (10و30) والارتيميا بمعدل (100و200) فردا/مل (للمستوى المنخفض والعالى على التوالى) وكانت المعاملات على النحو التالى:

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.

ولقد أظهرت النتائج أن هناك اختلافات ذات دلاله إحصائية ($P>0.05$) لمعدل الاعاشه حيث تحسنت مع اسخدام المستويات العالية من الروتيفر والارتميا. في حين وجد ان كفاءة النمو ليرقات اسماك الدنيس (الطول والوزن ؛ معدل النموالميومى فى الطول والوزن ومعدل النمو النوعى) تحسنت بشكل كبير ($P>0.05$) مع زيادة مستويات التغذية على الغذاء الطبيعي. ونستنتج من هذه ا لدراسه اهمية استخدام الغذاء الطبيعى بمستويات عالية ليرقات الدنيس حيث يجعلها متاحة امام اليرقات فى هذه المرحلة من العمر.