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FACTORS AFFECTING SWIM-BLADDER INFLATION, SURVIVAL, AND GROWTH PERFORMANCE OF GILTHEAD SEABREAM SPARUS AURATA LARVAE: (1) ROTIFERS *BRACHIONUS PLICATILIS* CONSUMPTION.

ZAKI[1*] M.A.; A.A. NOUR[2]; M.M. ABDEL-RAHIM[3] AND H.A.MABROUK[4]

[1-] Animal and Fish Production Dept., Faculty of Agriculture, Alex.Univ, Egypt.
[2-] Animal Production Dept., Faculty of Agriculture, Damanhour, Alex.Univ., Egypt.
[3]-Marine Hatchery, Km 21, Alex., General Authority for Fish Resources Development, Egypt.
[4-]National Institute of Oceanography and Fisheries. Qayed bay, Alexandria ,Egypt.

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ABSTRACT

Four densities of rotifers, Brachipnus plicatilis (4, 8, 12, and 16 pcs/ ml) were fed to gilthead sea bream larval for 21 days in fiberglass tanks. Each treatment was replicated in three tanks ($4m^3$ water volume/ tank) in greenhouse. Fish larval, two days old and 2.5 mm length were stocked at density 87±3 larval / liter of water. Each tank was supplied with continuous aeration, constant temperature (18^0C) and 12 hrs lights daily. Water quality criteria were within the optimum levels required for rearing fish larval. The results showed that the actual daily consumption of rotifers were 3.33, 5.20, 8.58, and 9.12 pcs /ml/day from the concentrations 4, 8, 12, and 16 pcs/ ml, respectively. Sea bream larval growth in length and the specific growth rate (SGR%) were significantly ($P \leq 0.05$) increased with increasing the rotifer density. The results showed that swim bladder inflation (%) significantly ($p \leq 0.05$) increased with increasing of rotifer density from 4 to 12 rotifers /ml, however there were no significant differences between 12 and 16 rotifers /ml. Survival rates of fish larvae at the end of the experiment were 24.4, 31.4, 44.25, and 47 % for rotifer densities 4, 8, 12 and 16 pcs/ ml, respectively.

From the present results it could be concluded that 16 rotifers/ml is the optimum density required for gilthead sea bream Sparus aurata larvae and about 1066.8 pcs of rotifers were required for each 1mm increase in larval length during the period from the 2nd to 21 rd days of age in the commercial hatchery,21 km, Alexandria .

INTRODUCTION

Marine fish farming plays an important role in aquaculture; the increase in demand of aquatic products and difficulties in fishing encouraging the development of marine fish farming (Yildiz and Sener, 1997). In Egypt, as in the other Mediterranean countries, sea bass and sea bream farming constitutes an important part of marine aquaculture especially along the coastal zones of both Red and Mediterranean.

One of the most important factors affecting survival and growth of sea bream and sea bass larvae in the hatcheries is the quantity and the quality of food during the most critical period before weaning of larval fish (the first three weeks) until the complete formation of swim-bladder. 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),

*Corresponding author

and 3-the larval stage (days 25-35).One of the most important food during this period is the rotifers *B. plicatilis* (Dhert,1996).This kind of natural food is very suitable for feeding many species of marine fishes, such as sea bream *S.aurata*, sea bass *Dicentrarachus labrax*, halibut , turbot, sole, red sea bream, flat fish, clown fish , Japanese blue crab and prawn *P. 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 these animals at very high densities up to 2000 animals / ml (Reitan *et al*., 1994) ; 2- tolerate a wide range of a suitable conditions ; 3- had high reproduction rate; 4- planktonic nature (Dhert, 1996) , 5- had many sizes that makes it suitable for many species and ages of fish and shrimp larvae, and 6- it can be cultured on cheap formulated feeds Dhert, 1996 high algal densities, and /or baker, yeast with added marine oil (Olsen *et al*., 1993; Rainuzzo *et al*., 1994; Reitan *et al*., 1997). Mass production and preservation of marine rotifer resting eggs is the current and hopeful trend (Dhert *et al*., 1995; Hagiwara *et al*., 1995).

The present study aims to: determination and ensuring the positive effect of the quantity of rotifer on survival rates swim-bladder inflation and growth rate of gilthead sea bream larvae, determination of the optimal concentration of rotifer and the actual consumption rate of rotifer, as well as increase the efficiency of the Egyptian marine hatcheries.

MATERIALS AND METHODS

Experimental Facilities:

This experiment was conducted in the Marine Fish Hatchery, 21 Km, and General Authority for Fish Resources Development, Ministry of Agriculture, and Alexandria, in two greenhouses for three weeks. Twelve circular fiberglass tanks, each containing 4m^3 water and equipped with 6 airlines were used. The tank walls were opaque, while the was maintained at 12 hr light: 12 hr dark. Light intensity was increased from 80 lux in the first week to 160 lux in the second week and 200 lux in the third week by installing 100 watt lamp over water surface; besides the fluorescent lamps bottoms were white. Photoperiod hanged in the two greenhouses.

Seawater (35 ppt salinity level) used in the present experiment was pumped through from a sand filter and passed through clothes, filter (200 micron) before being interred to the experimental tanks. Dissolved oxygen (DO), Temperature and pH in each tank were measured once daily at 8: 30 am. Water exchange rates were 20, 40, and 60 % during the first, second and third week, respectively. Each tank, equipped with stand pipe fitted with nylon screen (100 micron) to prevent the rotifer from escaping. Gilthead sea bream larvae used in this experiment were artificial produced from matured brood stocks (4 years age with an average weight of 750-1000 gm). Fertilized eggs were incubated at hatching tanks with a total number of 450.000 eggs / tank (4m^3 each). Two days old newly hatched larvae were stocked in the experimental tanks at a density of 87±3.0 larvae /m/. These larvae were kept in the dark for three days (Oceanic Institute, 1995). Then light intensity increased gradually to the previous limits mentioned before. Tanks were siphoned once every day. A floating oil trap removes the oil film on the water surface.

Feeding System:

Tanks were fed with micro algae *Nannochloropsis oculata* as the main food for rotifers, at a density of 500,000 cells/ml during the first two days of this experiment and 300,000 cells / ml until the end of the experiment. Rotifer *Brachionus plicatilis* added and controlled daily at four tested

densities (4, 8, 12, and 16 pcs of rotifer / ml). Eight samples of water were taken daily at 10 am from different sites of the tanks (representative sample) to determine the density concentration of rotifer before adding fresh (newly harvested rotifer) to adjust the required density of rotifers. The daily consumption of rotifers is determined for each tank by subtracting the residual density of rotifers in a day from the density of rotifer in the previous day at the same time (after 24 hrs). Algae *Nannochloropsis oculata* and rotifer *Brachionus plicatil*s were produced according to the manual prepared by Oceanic Institute (1995). Rotifers were washed for 10 minutes before feeding larval rearing tanks.

Zoo-technical methods:

Larval were sampled on the 7th, 14th and 21st days from different treatments in the tanks using 1-liter beaker after aeration was stopped. Swim-bladder inflation, total body length, and rotifer's uptake were monitored every week. Total body length was measured using a sample of 30 pcs. Of larva while a sample of 50 pcs. was used to detect the percentage of swim-bladder inflation. Fish survival rate was monitored at the end of the experiment not every week to avoid fish stress resulted from sampling. This experiment lasted 22 days from the 2nd day after hatching to day 23. Measurements mentioned were calculated according to the following formula:-

Specific growth rate (SGR) in length = 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)

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

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)

Swim-bladder inflation (%) = 100 (LS /TL)

Where: LS: no.of larvae having swim-bladder

TL: total no.of larvae tested

Rotifer's uptake = FD - ID

Where: FD: density of rotifer (pcs/ml) in a day

ID: density of rotifer (pcs/ml) in the previous day at the same time.

Water quality:

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

Statistical Analysis:

Statistical analysis was performed using (SPSS Version 10 program) and treatments were evaluated at the 0.05 probability. Analysis of variance, one – way ANOVA was used to evaluate the effect of rotifer density on total body length, SGR in length, daily consumption and survival rates of sea bream larvae. The differences within treatments were determined using LSD new were used at a 0.05 probabilities (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Water quality:

The means and range of the water quality parameters were within suitable limits for larval rearing tanks: salinity (35 ppt); temperature, 17-19.2 °C; dissolved oxygen DO2, 6.8-9.1 ppm, and pH, 8.2 - 8.45. Similar parameters have been reported by (Akatsu *et al.*, 1983).

Swim-bladder Inflation:

Table 1.Shows a high significant ($P<0.01$) effect of the density of rotifer on swim-bladder inflation (S.B.) during the first, second, and third weeks with significant

differences (P<0.05) between 4,8and 12 pcs of rotifers /ml and no significant difference between 12 and 16 pcs/ml.The final percentage of swim bladder inflation after three weeks was 46.7, 62.7, 89 and 90.5 % at 4, 8, 12 and 16 paces of rotifer/ ml, respectively. Fig. 1. Shows the weekly increase in swim-bladder inflation under the four tested treatments containing also various densities of rotifers.

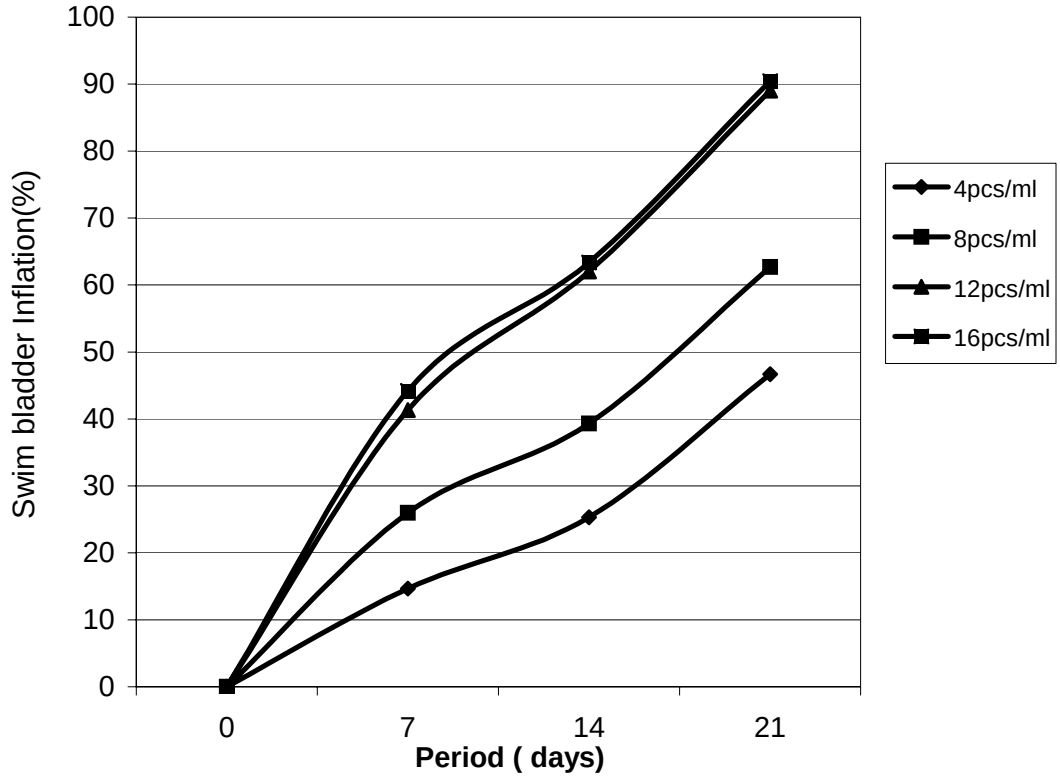

Fig.1. Effect of feeding various density of rotifer on swim bladder inflation on gilthead sea bream larvae *Sparus aurata* reared in commercial fish hatchery, Alexandria.

Table 1: Effect of feeding various densities of rotifers *Brachionus plicatilis* on swim bladder Inflation (%), growth performance, survival (%) and rotifers consumption of gilthead sea bream *Sparus aurata* larvae reared in commercial fish hatchery.

Items	Density of rotifers (pcs /ml)				Average*
	4	8	12	16	
Initial length (mm)	**2.5**	**2.5**	**2.5**	**2.5**	**2.5 ± 0.2**
Swim-bladder Inflation (%)					
1st Week	14.67^{c}	26.00 ^{b}	41.33^{a}	44.20^{a}	30.40 ± 3.83
2nd Week	25.33^{c}	39.33 ^{b}	62.00^{a}	63.35^{a}	46.06 ± 5.07
3rd Week	46.67^{c}	62.67 ^{b}	89.00^{a}	90.45^{a}	70.54 ± 5.88
Final Length (mm)					
1st Week	03.13^{c}	3.32 ^{bd}	3.38^{ad}	3.53^{a}	3.34 ± 0.05
2nd Week	03.88^{c}	4.25 ^{b}	4.60^{a}	4.75^{a}	4.33 ± 0.11
3rd Week	05.21^{c}	5.54 ^{b}	5.86^{a}	6.04^{a}	5.60 ±0.11
SGR in length (%/day)					
1st Week	03.21^{c}	4.05 ^{bd}	4.31^{ad}	4.93^{a}	4.13 ± 0.05
2nd Week	03.07^{c}	3.53 ^{b}	4.40^{a}	4.24^{a}	3.81 ± 0.11
3rd Week	04.21^{c}	3.79 ^{b}	3.46^{a}	3.43^{a}	3.72 ± 0.11
SGR in Length (%/day)	03.50^{c}	3.80 ^{b}	4.06^{a}	4.20^{a}	3.89±0.09
Survival rate[1] (%)	24.40^{b}	31.40 ^{b}	44.25^{a}	47.0^{a}	35.83 ± 3.11
Malformations (%)	24.00^{a}	18.00^{ab}	13.00^{bc}	10.0^{c}	16.25 ± 2.1
Rotifer's Uptake(pcs / ml / day)					
1st Week	01.33^{b}	2.75 ^{b}	5.47^{a}	5.82^{a}	3.66 ± 0.72
2nd Week	03.00^{b}	4.67 ^{b}	7.67^{a}	7.85^{a}	5.61 ± 0.70
3rd Week	03.33^{b}	5.20 ^{b}	8.58^{a}	9.15^{a}	6.33 ± 0.91
Rotifer Productive Factor (RPF) of (Riot. / 1mm incr) in length	945.2 ^{a}	1062.3 ^{a}	1169.5^{a}	1101.8^{a}	1066.8±51.4

Means (± S.E) in the same row with different superscripts are significantly different ($p \leq 0.05$)
* Means (± SE) in this column having one or two astric are significantly different at ($p \leq 0.05$.
Swim-bladder Inflation (%) = 100 (no. of larvae having Swim- bladder / Total no. of tested larvae)
SGR = 100 (ln Final length - ln Initial length.) / Period (days)
Survival = 100 (Final Number of larvae/ Initial Number of larvae
[1] Survival rate in each treatment was estimated only at the end of the experiment in order to avoid Any stress on fish during the experimental period.
Rotifer's Uptake= concentration of rotifer (pcs/ml.)- Conc. of rotifer (pcs/ml.) in the previous day at the same time.

There are many factors (e.g. feed type, feeding quantity, quality temperature, salinity, light intensity, photo period, water exchange, stress, aeration level) factors

affecting the formation of functional swim-bladder inflation in fishes (Al-Abdul- Elah 1990, Barnabe 1990, Al-Abdul Elah and Ross, 1993). The data reported that a highly correlation between the concentration of rotifer and the daily uptake of rotifer. However, there are few studies dealing with the influence of concentration of rotifer on the formation of functional swim bladder for gilthead sea bream hatcheries (Fieldera *et al*., 2002). Swim bladder inflation for snapper, *Pagrus auratus*, and larvae. Was best (80-100%) in an intermediate photoperiod of 12L: 12D at 9 dah. By 15 dah, although the percentage of larvae with inflated swim bladders had increased in all treatments, swim bladder inflation in 12L:12D was 1.3 and 2.0 times greater than that of larvae in 18L:6D and 24L:0D, respectively,(Fieldera *el al*., 2002) .Larvae showed the highest percentage of non-inflation exhibited the lowest growth performance (Crespo *et al*., 2001).

Growth Performance:

The results in Table (1) shows that, there were significant differences between treatments 4, 8 and 12 rotifers /ml in body length but the differences were insignificant between density 12 and 16 rotifers /ml for all treatments is shown in Fig. 2. As a result, the differences between the calculated SGR % in length under the tested treatments were only insignificant between ($p \leq 0.05$) the densities12 and 16 rotifer /ml. Values of SGR%, after three weeks of weaning, were 3.5, 3.8, 4.06 and 4.2 SGR %/day) for rotifer density or 4, 8, 12, and 16 /ml, respectively (Fig. 3). Flobela *et al*., (1996) studied the effects of the enriched rotifer with different materials from the first feeding on the growth and percentage of swim-bladder inflation of *Sparus aurata*. The results showed that the best growth in length was performed when rotifer enriched with Frippak booster. The presences of swim bladder positively increase growth performance. Crespo *et al*., (2001) found that the batch of larvae showing the highest percentage of non-inflation exhibited the lowest growth performance.

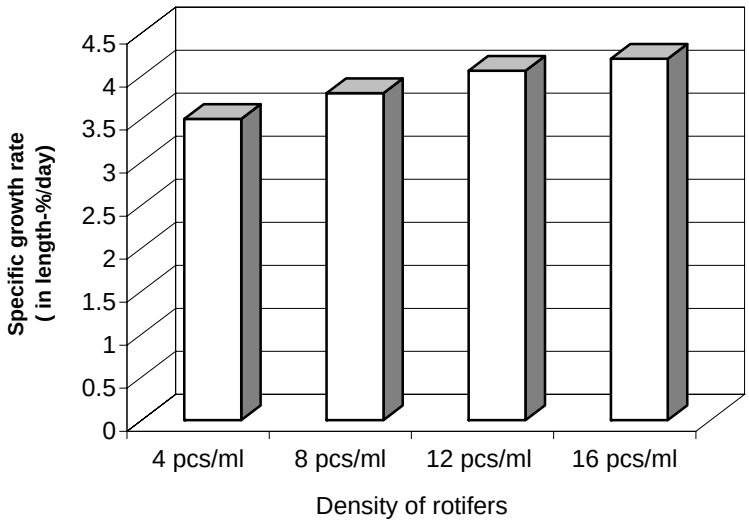

Fig.3: Effect of feeding various density of rotifer on the specific growth rate in length of gilthead sea bream larvae *Sparus aurata* reared in a commercial fish hatchery, Alexandria.

Rotifer's consumption and Utilization:

One of the most important results in the present study is measuring the actual rate of daily consumption of rotifers during the experimental period (3 weeks) and for all treatments. This result will give good indications about the destiny of rotifer that added daily in the tanks. These results reveal a significant difference of the rotifer's uptake between weeks 1, 2, and 3 with increasing trend in the rate of rotifer consumed (Table 1and Fig. 4). The differences between treatments (4 and 8 rotifer/ml) and (12 and 16) rotifer/ml. were significant ($p \leq 0.05$) without any significant differences between both 4 and 8, or 12 and 16 rotifer/ml. The best treatment was at 16 pcs rotifer / ml. The nutritional quality of the rotifer will definitely affect larval survival, swim-bladder inflation (Abdul-Elah and Ross, 1993. Flobela *et al.*, 1996). Rotifer can be boosted or enriched with fatty acid profile by application of emulsified or micro-articulated products in HUFA (Sorgeloos, 1994). The recent studies confirmed the importance of DHA and DHA/ EPA ratios in promoting growth, survival, and larval quality e.g. reduced malformations, stress resistance and improved pigmentation (decreasing albino ratio).
(Sorgeloos, 1994; Dhert *et al*., 1998).

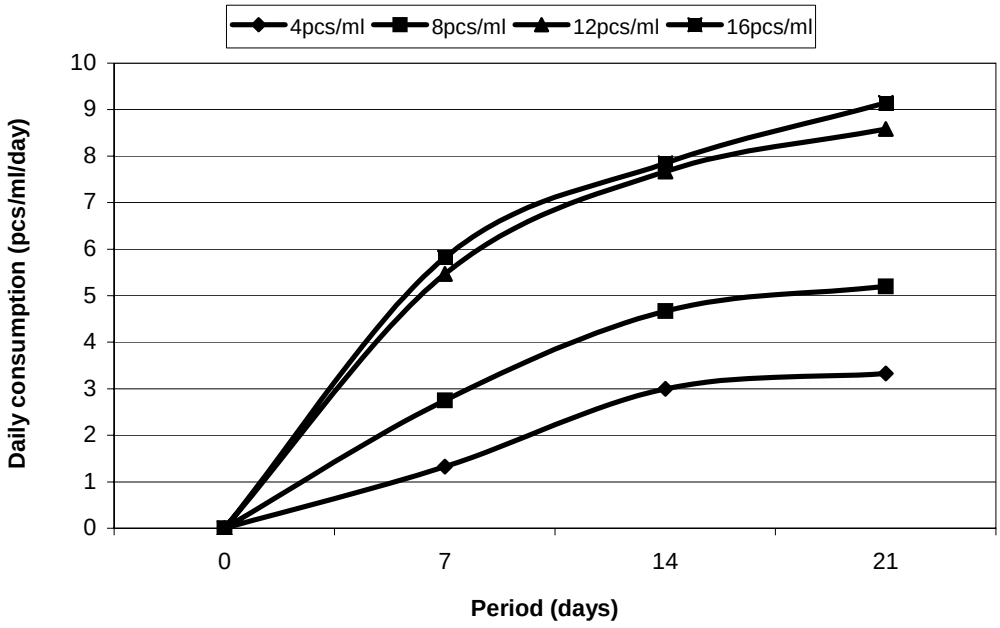

Fig.4. Effect of feeding various density of rotifer on the daily consumption for gilthead sea bream larvae *Sparus aurata* reared in a commercial fish hatchery, Alexandria.

Flobela *et al.*, (1996) studied the effects of the enriched rotifer with different materials from the first feeding on the percentage of swim-bladder inflation of *Sparus aurata*. The results showed that the best percentage of swim-bladder (85- 98%)

was formed with rotifer enriched with Chlorella sp. The same trend was observed in our experiment .The rotifer was enriched by *Nanno chloropsis oculata* . The recent trend in marine fish hatcheries all over the world is bolstering rotifer with selected probiotics and/ or antibiotic. The application of this technique results in improving the quality of rotifer, reducing bacterial contamination in fish larvae, and increasing fish survival rate (Sorgeloos, 1994). Improving the percentage of swim bladder Inflation will improve fish abilities for swimming, hunting, feeding, and growing.

Relationship between rotifer consumption and larval length:

Table (1) and Fig (5) clearly show the relationship between the daily consumption uptake of rotifers (DUR, pcs/ml/day) and total body fish length (L, mm) of fish larval during the present study. Figure 5 supported with the optimum equation (the inverse equation which has the basic formula: Y= b0+(b1/L) where Y is the daily uptake of rotifers (DUR), L is the total fish length, and b0 and b1 are constants. According to this equation (DUR= 16.0534-(39.074/L) with r^2 value of (0.978) and the significance of the obtained correlation was 0.011. Unfortunately, there is not available data about this point. However, Lizawa (1983) linked between the daily quantity of rotifer ingested (D.Q.R) and the total body length of European sea bass larvae using the following equation: D.Q.R = 2.39 x 10-4 x L3.99.

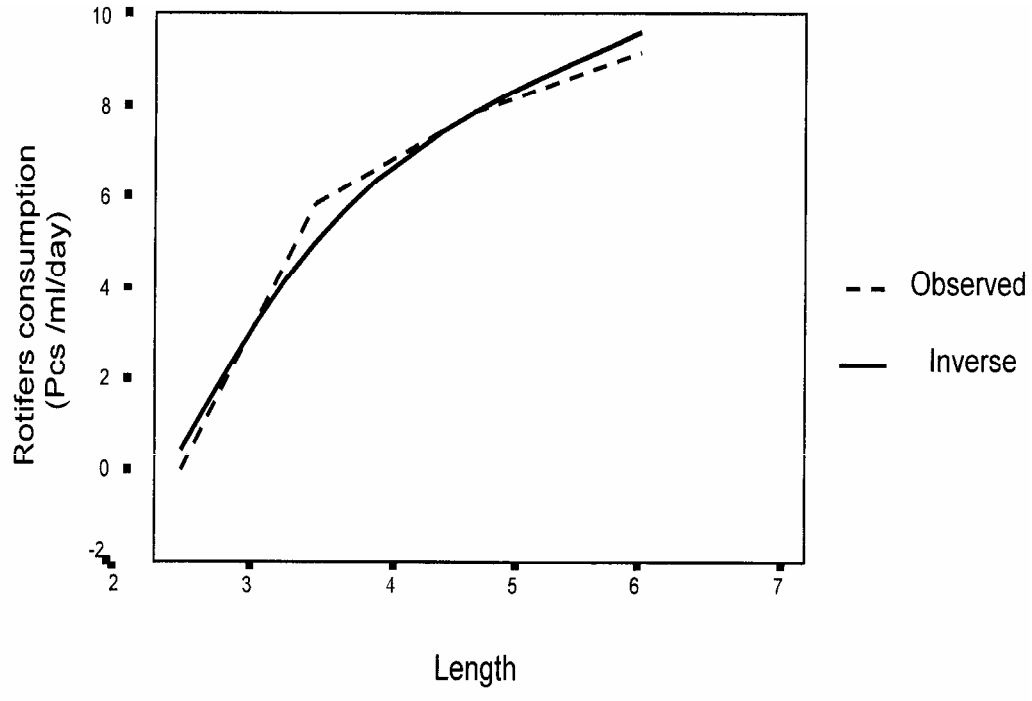

Fig.5: The relationship between the consumption (uptake) of rotifer (DUR) and total body length (L,cm) of gilthead sea bream (*Sparus aurata*) larvae reared at density of 2 and 16 pcs/ml in a commercial fish hatchery.

The inverse equation at 12 and 16 roti.fers/ml is DUR= 6.05-(39.07Ll)and DUR/15.62-(37.60/L), respectively.
Where DUR = daily uptake consumption of rotifers
L= length (mm), RSQ= 0.978and 0.961 respectively

Rotifers productive factor (RPF) is a parameter designed to estimate the actual number of rotifer, which is necessary to increase 1 mm of total larval length. The number of consumed rotifers did not different significantly ($P<0.05$) with the density of rotifers. This result is very important in determining the optimum density of rotifers to get the best growth and survival rate of fry during this critical period of larval age and the total number of rotifers for gilthead sea beam.

Fish survival rate:

The most important factor in evaluating fish hatcheries is larval survival rate. The fish density of rotifers significantly ($p\leq0.05$) increased fish survival rates (Table 1 and Fig.6). The lowest survival rates were obtained by low feeding densities of rotifer (4 and 8 pcs /mp). While, the survival rates were doubled in the high feeding density (12 and 16 pcs/ml) comparing with these in the lowest densities (Table 1 and Fig.6).

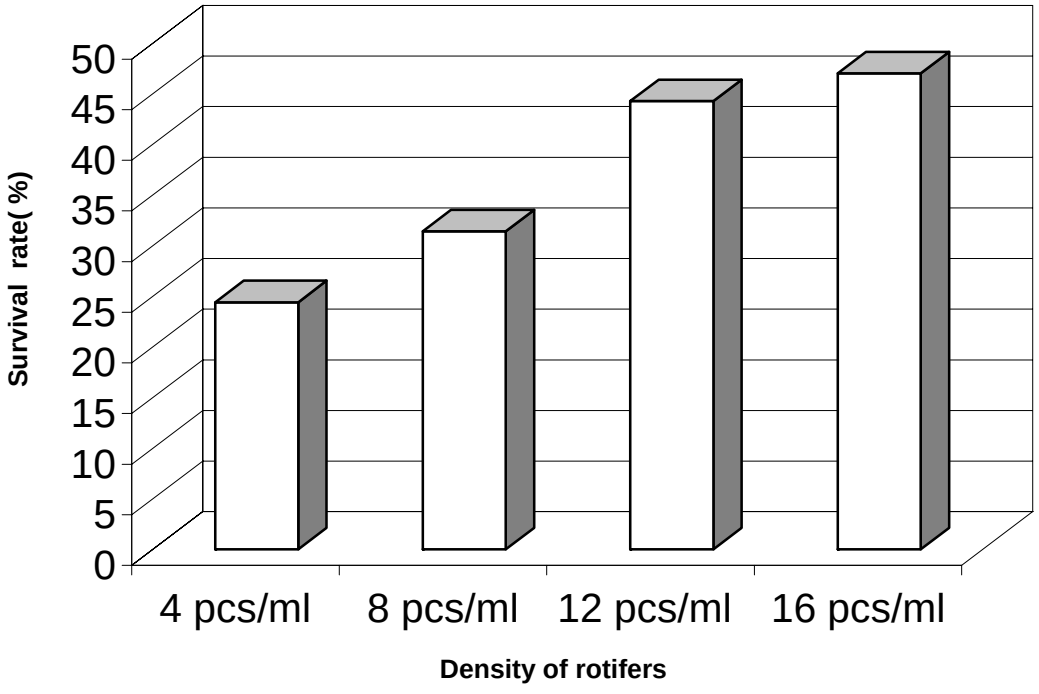

Fig.6: Effect of feeding various density of rotifer on the survival rate of gilthead sea bream larvae Sparus aurata reared in commercial fish hatchery, Alexandria.

Larval malformation:

Values of the percentage of abnormalities or malformations were 24, 18, 13, and 10 % at 4, 8, 12, and 16 rotifer/ml, respectively (Table 1 and Fig. 7) with significant ($P \leq 0.05$) differences between treatments. The result of our experiment clearly shows the importance of larval fish nutrition to reduce the percentage of fish skeletal malformation (lordosis). The relationship between the presence of inflated swim bladder and larval deformities has been stressed (Andrades *et al*., 1996; Goolish and Okutake, 1999; Kihara *et al*., 2002; Gavaia *et al*., 2002). Yih and Han (1996) mentioned two types of skeletal malformations, lordosis (10-21%) and branchyospondyliosis (1-4%). Garcia (1997) obtained a high mortality rate (70%) of the abnormal fish. Increasing stocking density could increase the percentage of malformations (Koumoundouros *et al*., 1997; Yih and Han, 1996). Lordosis is correlated with absence or malfunction of the swim bladder. However, swim bladder abnormalities do not completely explain the occurrence of lordosis. The high incidence of malformations may reflect culture problems due to rearing and/or feeding conditions that affect skeletal development. Gavaia *et al*., (2002).

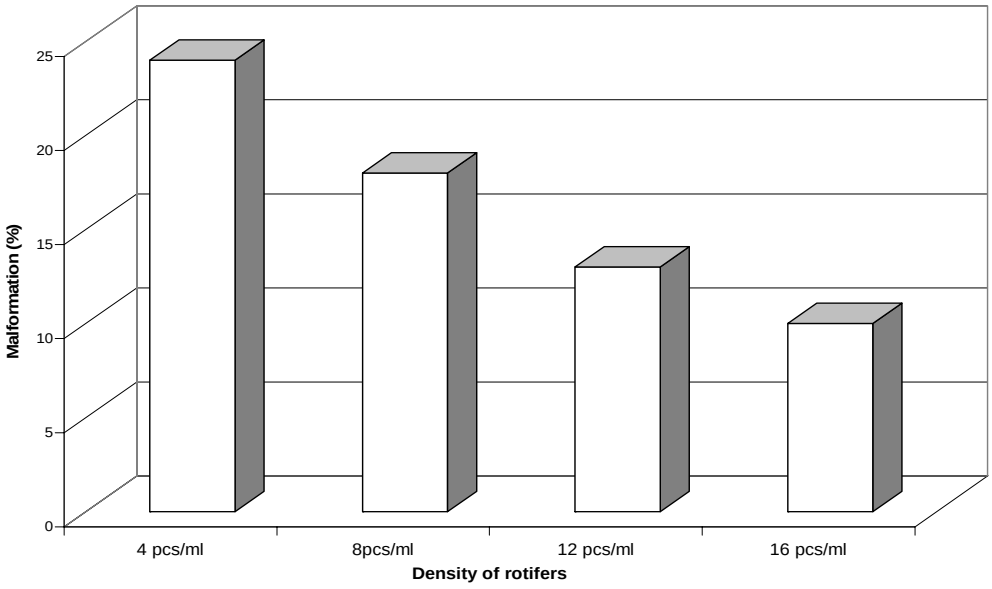

Fig.7: Effect of feeding various density of rotifer on malformation (%) of gilthead sea bream larvae Sparus aurata reared in commercial fish hatchery, Alexandria.

Finally it could be recommended to use 16 rotifer/ml of water for feeding gilthead sea bream *S.aurata* larvae from the 2nd day of age until adding artemia at the 2 rd day of age. This to ensure formation of functional swim-bladder inflation, which improves the abilities of larval fish for swimming, hunting, feeding, growing, and preventing deformities in fish fry.

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