EFFECT OF LIVE FOOD ON SURVIVAL RATE AND GROWTH PERFORMANCE OF GILTHEAD SEABREAM Sparus aurata LARVAE.

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ABSTRACT

This experiment was carried out in order to study the effect of rotifers, *Brachionus plicatilis*, and encapsulated *Artemia nauplii as a* live food on survival rate and growth performance of gilthead sea bream, *Sparus aurata*, larvae. Gilthead sea bream larvae (20 days old) with 7.3 ± 0.20 mm/pce body length and 5.4 ± 0.10 mg/pce body weight were stocked in eight white fiberglass tanks (each of 1 m³ volume) at a density of 1,200-larvae/ tank. Four treatments were tested as following, 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. Low and high rotifers were 5 and 25 (pcs/ml), respectively. Low and high *Artemia* were (50, 100, and 200 pcs/ml) and (250, 500, and1000 pcs/ml), during the periods 1st-8 th, 9 th -16 th and 17 th -24 th days of feeding, respectively.

The results showed that survival rates of , S. aurata, larvae significantly (P≤ 0.05) improved with the application of the experimental program by feeding mixture of high levels of rotifers and Artemia. Feeding a mixture of higher levels of rotifers and Artemia increased survival rate (48.96 %), however, the lower levels of both resulted in lower survival rate (12.17) % of , S. aurata larvae. Other intermediate treatments of HRLA or LRHA resulted in improvements in survival rates (19.08 and 32.21) %

respectively) of ,S. aurata ,larvae.

Growth performances of, *S.aurata*, larvae (gain in length and weight; average daily gain in length and weight; and specific growth rate, SGR %) were significantly ($P \le 0.05$) 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 the growth performances criteria of, *S. aurata*, larvae than rotifers. Finally it could be recommended a suitable live food program containing a mixture of rotifers and *Artemia* to improve the survival rates of, *S. aurata*, and larvae.

Keywords: Rotifers, Artemia, gilthead sea bream, survival

INTRODUCTION

Seed production and larval rearing still form the bottleneck 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) specially 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 rotifers, Brachionus plicatilis, has been most widly used as essential food source in raising marine environment (Lubzens, 1987; Dhert et al., 1994). Zaki et a., 2004 showed that the concentration of rotifers per ml of water was optimum (16 rotifers /ml) for feeding gilthead sea bream S. aurata larvae. This concentration is a good 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.

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, Artemia . 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 reaing of larval seaabream , S. aurata :a)the end of the pre- larval stage (day 3-4);b) the endo exotroph stage (day 8-12);c)the larval stage (day 25-35). More than 99 % of the fry were losted during these phases (Dhert et al., 1998)and mean survival rate 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 techniques 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 phase (Maneewong *et al.*, 1986 a,b).

The aim of the present work was to improve the survival rates and growth performances 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 of the General Authority for Fish Resources Development, (GAFRD), Ministry of Agriculture in order to study the effect of rotifers, Brachionus plicatilis, and encapsulated "Artemia 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.

Two types of live foods rotifers, *B. plicatilis*, and encapsulated *Artemia nauplii* were tested in four combinations, of treatments as following 1) low level of rotifers and low *Artemia* (LRLA); 2) high level of rotifers and low levels of *Artemia* (HRLA); 3) low level of rotifers and high levels of *Artemia* (LRHA); and 4) high level of rotifers and high levels of *Artemia* (HRHA), respectively. Low and high levels of rotifers were 5 and 25 pcs/ml), respectively during the period of experiment. *Artemia* low (50,100, and 200 pcs/ml) and high (250, 500,

and1000 pcs/ml), respectively during the periods 1st-8th, 9th -16th and 17th -

24th days of the feeding experiment, respectively.

Fish larvae used in the present experiment were produced from tankmatured S. aurata brood stocks (4 years old and average weight of 750-1000 gm). Fish broad stocks were previously spawned artificially at water temperature ranges from 16-18 °C using LHRH-hormone pellets. Fish larvae (20 days old) with average body length of 7.3 mm/pce and average body weight of 5.4 mg/pce were collected from a high-density larval rearing tank in the neighbor larval greenhouse using water bucket at night. Larvae were stocked in the experimental tanks at a density of 1200 pcs of larvae /tank (1.2 pcs/liter) on 15 may 2003.

Seawater (35 ppt) was pumped via a sand filter and passed through clothes filter (200 micron) before being interred to the tanks. Water exchange rate was 40, 50, and 60 %daily during the period 1st-8 th, 9 th -16 th and 17 th -24 th days of the experiment, respectively. Each tank equipped with standpipe fitted with Nylon screen (100 micron) to prevent the rotifers from escaping. Photoperiod was maintained at 12hr light: 12hr dark (12L: 12D). Light intensity during the experimental period was 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 oculatawas added to rotifers rearing tank at a density of 200.000 cells / ml during the experimental period. Rotifers Brachionus plicatilis had grown on micro algae Nannochloropsis oculata according to the manual of Oceanic Institute, (1995). Rotifers were washed for 10 minutes before adding to larval rearing tanks.

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.

At the end of the experiment (9 June, 2003), samples of fish (50 pcs) were weighed and the average total lengths and weights in each treatment were measured to calculate the final, weight, average daily length and gain, specific growth rate (SGR % in length and weight) and condition factors. Larval survival rate was calculated after 24 days through counting the total number of 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 (In FL - In 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 (In FW - In 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 = Weight (gm)/length³ (cm

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 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.

RESULTS AND DISCUSSION

The averages of water quality criteria for gilthead sea bream , *S. aurata* , larval rearing tanks Throughout the culture period were as following: salinity 35 ppt; temperature 19.6 °C; dissolved oxygen 6.9 ppm DO₂, and pH 8.02. Similar parameters have been reported by several authors (Zaki *et al.*, 2004 and Nour *et al.*, 2004).

Results in Table (1) showed the effect of four different live food treatments from different combinations of rotifers and *Artemia* (LRLA, HRLA, and LRHA and HRHA) on survival and growth performance of *S.aurata* larvae. Survival rate (%) of *S.aurata* larvae were significantly (P≤0.05) increased from 12.17 % with LRLA to 48.96 %with HRHA .Higher live food concentrations from rotifers or *Artemia* resulted in a better survival rate of *S.aurata* larvae than control group (LRLA). However, the significant role of *Artemia* over rotifers as live food was clearly observed in the differences between the 2nd (19.08) and 3rd (32.21%) treatment respectively.

Growth performance of *S. aurata* larvae fed different combinations of live food (LRLA, HRLA, LRHA and HRHA) showed a significant differences (P≤0.05) in larval length and weight; gain in length and weight; average daily length and weight and specific growth rate, SGR %, in length and weight (Table 1).Growth in length (Table 1 and Fig.1) showed the superiority of *Artemia* (treatment HRLA) as live food for *S. aurata* larvae over rotifers (treatment LRHA). While, (treatment HRHA) higher concentrations of mixture of rotifers and *Artemia* resulted higher growth of length and weight (Table 1 and Fig.2).

The feeding strategy during the preweaning phase is based on standard feeding scheme in witch enriched rotifers are replaced by newly hatched *Artemia*.

Table (1) Effect of 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° | 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° | 14.30 ^b | 15.65 ^a |
| Length gain (mm/pce) | 4.20 ^d | 5.50° | 7.05 ^b | 8.35° |
| ADL (mm/pce/day) | 0.18 ^d | 0.23° | 0.29 ^b | 0.35° |
| SGR in length (%/day) | 1.89° | 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° | 18.15 ^b | 21.95 ^a |
| Weight gain (mg/pce) | 5.45 ^d | 8.35° | 12.75 ^b | 16.55° |
| ADG (mg/pce/day) | 0.23 ^d | 0.35° | 0.53 ^b | 0.69ª |
| SGR in weight (%/day) | 2.91 ^d | 3.89 ^c | 5.05 ^b | 5.84ª |
| d) 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 = 50,100,and 200 pcs /ml during the periods 1st-8th, 9th -16th and

17 th -24 th days of the feeding experiment, respectively.

High Artemia = 250,500, and 1000 pcs /ml during the periods 1st-8th, 9th -

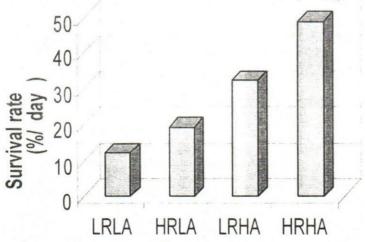
16 th and 17 th -24 th days of the feeding experiment, respectively.

Means in the same row with different superscripts are significantly different (p \leq 0.05)

SGR (%/day) = 100 (In Final length - in Initial length.) / Period (days)

Survival rate (%) = 100 (Final Number of larvae/ Initial Number of larvae)

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



Treatments

Fig. (1) Effect of live food on survival rate (%/day) for gilthead sea bream Sparus aurata.

LRLA: low rotifers and low artemia LRHA: low rotifers and high artemia

HRLA: high rotifers and low artemia HRHA: high rotifer and high artemia

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 periods before weaning of larval fish until the complete formation of swim-bladder. Dhert et al., (1998) mentioned that there are three main critical and sensitive phases of larval age 1-the prelarval stage (day 3-4), 2- the endo- exotroph stage (days 8-12), and 3-the larval stage (days 25-35). One of the most important food during this period is the rotifers, Brachionus plicatilis (Dhert, 1996). This kind of natural food is very suitable for feeding many species of marine fishes, such as sea bream, , sea bass, , halibut, turbot, sole, red sea bream, flat fish, clown fish , Japanese blue crab and shrimp (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 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., 1994b; 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).

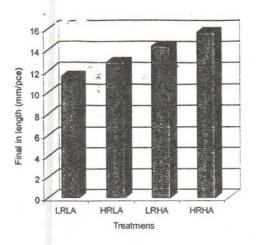
The results of Zaki et al., (2004) clearly show the importance of larval fish nutrition to reduce the percentage of fish skeletal malformation (lordosis). The relationship between the presence of inflated swim bladder and larval deformities has been stressed by several authors (Andrades et al., 1996;

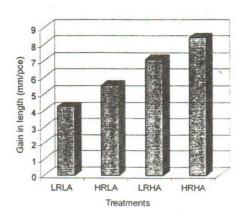
Goolish and Okutake, 1999; Kihara et al., 2002; Gavaia et al., 2002).

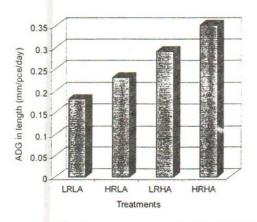
Yih and Han., (1996) mentioned two types of skeletal malformations, lordosis (10-21%) and branchyospondyliosis (1-4%). Garcia .,(1997) obtained a high mortality rate (70%) of the abnormal fish. Increasing stocking density could increase the percentage of malformations (Koumoundouros et al., 1997; Yih 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). The results of the present study clearly showed that S. aurata larvae prefer Artemia over rotifers Artemia nauplii are used extensively world-wide as live food for the larval stages of commercially important fresh water and marine fish species. The above findings suggest two possible modes of influence by Artemia nauplii on the ingestion ,digestion and assimilation of microdiets (MD) during co-feeding) : (1) the remote influence on MD ingestion by ingestion by visual and chemical stimuli and /or (2) the direct influence of the biochemical composition of Artemia nauplii on larval digestion and assimilation. Kolkovski et al., (1997) found that the MD ingestion rates in seabream larvae exposed to visual and or chemical stimuli generated by various concentrations of Artemia nauplii, increased up to 120% as compared to ingestion rates in larvae that were offered the MD alone . Moreover , both visual and chemical stimuli were found to work synergistically Larval acuity is considered vital for larval orientation and food recognition (Blaxter, 1968; Hunter, 1981) and mutual reinforcement of chemical and visual stimuli would enhance the success of early feeding .On the other hand , the magnitude of the influence of these stimuli decreased with larval age .Since visual acuity as well as neural and olfactory development increases with larval age (Blaxter, 1968), it is reasonable to assume that the dependence on rudimentary visual and chemical stimulation of the larvae by the prey will decrease as the larvae develops its hunting abilities (Fernald, 1993). Kolkovski et al., (1997) suggests that the influences exerted by Artemia nauplii on larvae to enhance the acceptance of a MD during co-feeding are tow fold :1) the remote influence the visual and chemical stimulation where the secreted free amino acids (FAA) glycine, alanine, arginine and the compound butane activate larval receptors stimulating appetite and orienting the fish to a food source ,(2) the influence of the biochemical composition of the live food on larval digestion and assimilation where Artemia enzymes and other factors may contribute to digestion directly or by activating zymogens or digestive hormones such as bombazine .Further studies ,however ,is still necessary in order to identify all the factors associated with Artemia nauplii that influence larval ingestion, digestion and assimilation .This knowledge and its application in MDs design is necessary pre-requisite to prepare MD which could be considered as a serious alternative to live food in marine fish larvae culture. Zaki et al .,(2004) concluded that 16 rotifers /ml is the optimum density required for gilthead seabream , S. aurata , larvae during the 1st 21 days of its 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 rates of *S. aurata* larvae were greatly improved when high levels of rotifers added together with *Artemia* (treatment 4). The positive effect of *Artemia* to *S. aurata* larvae during this stage of its life appear to enhance appetite of the larvae. Ganzon-Naret Fermin.,(1994) reported that delaying feeding of *Artemia nauplii* until days 15 resulted in slower growth rate of sea bass fed *Artemia nauplii* starting on day10. Further studies of the mechanisms and interactions between rotifers-larval, *Artemia* larval interactions at the various steps of the larval feeding process should be given high priority in future research. The present results clearly showed 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 and Fig.4).

From the present results it could be recommended the use of a live food mixture of rotifers and *Artemia* for feeding githead Sea – bream larvae in the commercial hatcheries in order to increase its survival rates and growth performance criterias.







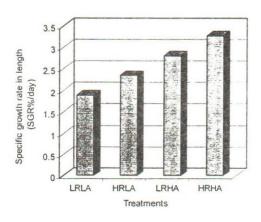


Fig. (2) Effect of Rotifer *Brachionus plicatilis* and decapsulated *Artemia nauplii as a* live food on growth performance in length a) final in length; b) gain in length; c) average daily gain (ADG) in length and d) specific growth rate (SGR) in length for gilthead sea bream *Sparus aurata*.

LRLA: low rotifers and low Artemia LRHA: low rotifers and high Artemia HRLA: high rotifers and low Artemia HRHA: high Rotifer and high Artemia

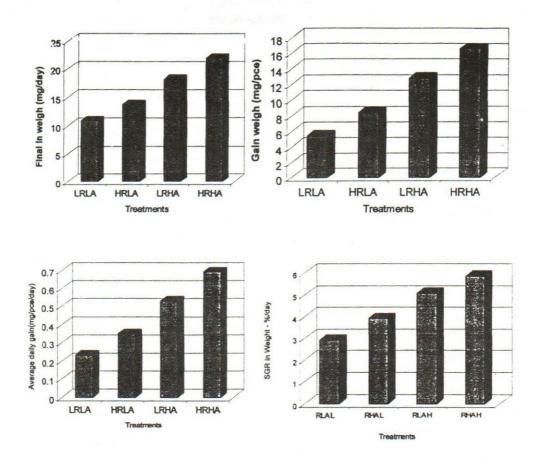


Fig. (3) Effect of Rotifer *Brachionus plicatilis* and decapsulated *Artemia nauplii as a* live food on growth performance in weigh a) final in weigh; b) gain in weigh; c) average daily gain (ADG) in weigh and d) specific growth rate (SGR) in weigh for gilthead sea bream *Sparus aurata*.

LRLA: low rotifers and low Artemia LRHA: low rotifers and high Artemia HRLA: high rotifers and low Artemia HRHA: high Rotifer and high Artemia

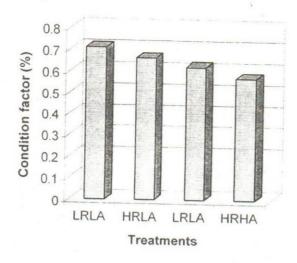


Fig. (4)Effect of live food on condation factors(%) for gilthead sea bream *Sparus aurata*.

LRLA: low rotifers and low artemia LRHA: low rotifers and high artemia

HRLA: high rotifers and low artemia HRHA: high rotifer and high artemia

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تأثير الغذاء الطبيعي على معدل الآعاشة وكفاءة النمو ليرقات اسماك الدنيس محمد أحمد عبدالله زكى قسم الانتاج الحيواني والسمكي - كلية الزراعة - جامعة الاسكندرية

تمت هذه التجربة في المفرخ البحرى بالكيلو ٢١ طريق إسكندرية مطروح من أجل دراسة تـــاثير التغذية بالروتيفرا والارتميا كغذاء طبيعي على معدل الأعاشة وكفاءة النمو ليرقات أسماك الدنيس .أستخدمت يرقات أسماك الدنيس بعمر ٢٠ يوم بمتوسط طول ٧,٣ ±٠,٠ مليمتر /قطعــة وزن ٥,٥±٠,١٠ مليجــرام اقطعة ، وتم تخذين هذه اليرقات في ثمانية تنكات مصنعة من الالياف الصناعية (حجم كل تانك امتراً وبكثافة ١٢٠٠ يرقة / تانك .وتم أختبار أربعة معاملات على مدى أربعة وعشرين يوما وهي كما يلي :-١) مستوى منخفض من الروتيفرا مع مستوى منخفض من الارتميا ، ٢) مستوى مرتفع مــن الروتيفــرا مــع مستوى منخفض من الارتميا ، ٣) مستوى منخفض من الروتيفرا مع مستوى مرتفع من الارتميا، ومســـتوى مرتفع من الروتيفرا مع مستوى مرتفع من الارتميا. المستوى المنخفض من من الروتيفرا ٥ قطعـــة / مــــل بينما المستوى المرتفع ٢٥ قطعة / مل أما المستويات المنخفضة من الارتيميا كانت (٥٠،١٠٠،٥٠ قطعة / مل) بينما المستويات المرتفعة كانت (٢٥٠،،٢٥٠ قطعة / مل) وذلك خلال الفُترات الغذائية باليوم (١-٨)، (٩-١١)، (١٢-٤٧)على التوالي.

أوضحت النتائج أن معدلات الاعاشة ليرقات أسماك الدنيس قد تحسنت بصورة معنوية مع بدايــة تطبيق برنامج التغذية التجريبي بأستخدام خليط من المستويات المرتفعة من الروتيفرا والأرتميا ، التغذية على الخليط من المستويات المرتفعة من الروتيفرا والارتيميا ادى الى زيادة نسبة الأعاشة ليرقات أسماك الدنيس الىي (٤٨,٩٦ %)، بينما التغذية على المستويات المنخفضة من الروتيفرا مع الارتميا نتج عنها أنخفاض معدلات الأعاشة ليرقات أسماك الدنيس الى (١٢,١٧ %) . متوسط المعاملات الاخرى رقم ٢،٢ نتج عنها تحسن في معدلات الاعاشة (١٩,٠٨ و ٢٢,٢١ على التوالي) ليرقات أسماك الدنيس كفاءة النمو ليرقات أسماك الدنيس قد زادت بصورة جوهرية بزيادة مستويات الغذاء الطبيعي سواء من الروتفرا أو الارتميا. وتشير النتائج الى أنه يمكن التغذية على مستويات مختلفة من الأرتميا الحية لرفع كَفاءة النمو ليرقات أسماك الدنيس عن التغذية على مستويات مختلفة من الروتيفرا. ويستخلص من هذا البحث أنه يمكن استخدام برنامج لتغذية يرقات أسماك الدنيس على خليط من الروتيفرا والارتميا كغذاء طبيعي لتحسين معدلات الاعاشة فسي المفرخات البحرية.