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# Effect of early weaning on growth and survival rate of gilthead seabream (*Sparus aurata*) larvae

**[1]Soltan, M. A. [2]Shaker, I. M. [1]Abd-Ella, M. M., [1]Soliman, A. S. M. and [2]Darwish, S. I.**
[1] Fac. Agric. Moshtohor, Benha University, Egypt
[2] Central Lab. for Aquaculture Research at Abassa, Sharkia Governorate, Egypt

## SUMMARY

The present study was carried out in the Marine Fish Laboratory (MFL), Faculty of Agriculture Saba Basha, Alexandria University, Egypt to study the possibility of early weaning of gilthead seabream (*Sparus aurata*) larvae. Therefore seabream larvae divided into different groups and weaned at 25, 27, 29, 31, 33, 36 and 39 days post hatching (dph). At the end of the experiment the mean wet weight and length of gilthead seabream larvae after 45 dph showed that, the late weaning (33, 36 or 39 dph) significantly increased the wet weight and length of larvae compared to larvae groups that early weaned (25 and 27 dph) and the same trend was also observed for larvae after 60 dph.

Weight gain of gilthead seabream larvae during the period of 45-60 dph ranged between 0.039 to 0.052 gm. The early weaning significantly decreased larvae weight gain (groups 25, 27 and 29 dph) while the late weaning (36 and 39 dph) significantly improved weight gain and the same trend was also observed during the entire experimental period (30-60 dph ).

During the entire experimental period the average larvae specific growth rate found to be 1.39, 1.60, 1.59, 1.54, 1.65, 1.70 and 1.71 for larvae groups weaned at 25, 27, 29, 31, 33, 36 and 39 dph, respectively and the differences between these means were significant.

At 30 dph survival rates for seabream larvae groups found to be 78.67, 86.67, 88.00, 85.33, 88.00 and 88.00. At 60 dph the average survival rates found to be 88.00, 90.33, 89.00, 86.33, 92.00, 99.00 and 89.33% for larvae groups weaned at 25, 27, 29, 33, 36 and 39 dph, respectively and the differences between these averages were significant.

Protein and fat content of gilthead seabream larvae at the end of the experiment ranged between 31.20-36.20 for protein and 2.10 -3.36% for fat, respectively. The obtained results indicated that the late weaning (36 and 39 dph) significantly increased each of protein and fat content of larvae when compared to the early weaning (25 and 27 dph).

## INTRODUCTION

To our knowledge, no information is available regarding weaning of seabream (*Sparus aurata*) larvae from live food, and how early weaning (i.e. before 25 dph) may affect the larval acceptance of microdiets and their growth and survival in relation to the development of digestive enzyme activities. Such knowledge is important for a better understanding of their digestive capacity for utilization of formulated diets and for optimizing larval rearing protocol of this specie.

In intensive production of most marine finfish species, weaning is a very critical period in which there is a gradual change from live prey to formulated diets (**Rosenlund *et al.*, 1997**). 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**).

Formulated diets can balance the nutritional composition of live food especially with respect to amino acids which are not easy to modify in live food (**Ronnestad *et al.,* 1999**). Live food may influence ingestion, digestion and assimilation of formulated diets. Live food influences digestion by stimulating endocrine responses (**Koven *et al.,* 2001**). In addition, co-feeding is expected to improve the nutritional condition of the larvae and might facilitate earlier transition onto dry feeds only (**Rosenlund *et al.,* 1997**).

The timing of co-feeding and the nutritional composition, palatability and digestibility of the artificial diets determine the success of weaning (**Faulk *et al.,* 2007**). **Kestemont *et al.* (2007)** indicated that the best weight gain of pikeperch larvae *(Sander lucioperca)* was found when co-feeding was started at 19 dph compared to 12 dph or 26 dph. Early co-feeding is beneficial, since it reduces the use of live food, which are cumbersome to produce and difficult to manipulate nutritionally (**Dhert *et al.,*1999**). Success of early co-feeding has been widely documented in various species such as tongue sole *Cynoglosssus semilaevis* (**Chang *et al.*, 2006),** striped bass *Morone saxatilis* (**Chu and Ozkizilcik, 1999)**, barramundi *L. calcarifer* (**Curnow *et al.,* 2006 a**), turbot *Scophthalmus maximus* (**Dhert *et al.,* 1999**), Atlantic halibut *Hippoglossus hippoglossus* **(Rosenlund *et al.,* 1997),** red drum *Sciaenops ocellatus* **(*Lazo et al.,* 2000 b)**, seabass *Dicentrarchus labrax* (**Rosenlund *et al.,* 1997**) and winter flounder *Pseudopleuronectes americanus* **(Khemis *et al.,* 2003**). However, the starting time for co-feeding is species-specific according to the maturity of the digestive system (**Cahu and Zambonino Infante, 2001).**

Using commercially available weaning diets, our aim of this study was to investigate the effects of early weaning strategy by graded substitute live food (*Artemia*) by artificial diets and larval performance using growth and survival as criteria. Therefore, The present study investigated the effect of weaning of seabream larvae (*Sparus aurata*) at different ages 25, 27, 29, 31, 33, 36 and 39 dph on growth, survival rate and the best feeding regime required for obtaining a good quality fish larvae of Seabream under the Egyptian conditions using different weaning ages.

Live food provides higher growth and survival rates in the early stages when compared with compound diets **(Hart and Purser**, **1996 and Blair *et al.*, 2003).** Therefore, live foods continue to be essential for the first feeding of marine fish larvae because they lead to increased feeding, stimulate enzyme secretion, and result in consistently good growth and survival.

Nevertheless, some disadvantages have been pointed out concerning the use of live foods: (1) the production and use of live foods is expensive **(Hart and Purser, 1996)**; (2) bacteria associated with zooplankton cultures can be detrimental to fish larvae and may act as vectors of diseases; (3) the nutritional values of live foods can be highly variable **(Evjemo, *et al.*, 2001 and Olsen, 2004)**; (4) nutritional quality of live foods is difficult to manipulate (5) metabolites from zooplankton increase the load on fish rearing systems; (6) fine-mesh outlet screens are required to retain zooplankton so low water flow rates are necessary to avoid screen blockages and resultant tank overflows.

For these reasons it is considered highly desirable to use artificial diets as early as possible in the rearing process. It appears that the main problems with artificial diets are their unacceptability to the larvae, low digestibility, and problems associated with lack of buoyancy and instability in water **(Jones *et al.,* 1993).**

There have been very few successful attempts to feed the larvae of marine fish on artificial diets from first feeding (**Walford *et al.,* 1991).** Until now only sea bass larvae have been reared successfully on an artificial diet from the time of mouth opening **(Cahu and Zambonino Infante, 2001).**

## MATERIALS AND METHODS

The present experiment was carried out in the Marine Fish Laboratory (MFL), Faculty of Agriculture Saba Basha, Alexandria University, Egypt. This work was done using newly hatched seabream fish larvae during the period of larvae rearing and feeding on live food.

**1. Sea water supply system:**

The water used for live food production and other activities in the MFL is transferred from the sea to the lab by car prepared for this purpose. The tank ($4\times1$ $m^3$) over the car is filled by pump and the water transferred to the ground storage tanks lab. After the precipitate the suspended particles in the transferred water was pumped to four reservoir tanks ($1m^3$ for each) over the Lab.

**2. Algae Production (*Nannochloropsis oculata*):**

Green micro algae *Nannochloropsis oculata* in the MFL is produced as feed for rotifer and water conditioner for larvae rearing to improve water quality and sustain rotifer alive. *N. oculata* is selected for its high productivity, the relative ease of its culture, suitability for rotifer culture and its tolerance of a wide range of salinities and temperature. *N. oculata* is high in nutritional value containing eicosapentanoic acid (EPA) and decosahexaenoic acid (DHA). It has been reported that these highly unsaturated fatty acids (HUFAs) are essential for the growth and development of finfish larvae (**Tamaru *et al* 1991**)

The algae culture starts with laboratory flask culture and up scaled gradually to outdoor mass culture. The outdoor culture is conducted in glass aquaria with a fiber glass cylindrical tank prepared to provide the aquaria with continuously algae flow. In the facilities of MFL, algae production consists of different stages as follows:

Maintenance and stock culture in 250 ml flasks in Indoor algae culture, and in Outdoor algae culture, starter culture: In 2L bottles and 10L polyethylene bags, intermediate culture: In a fiber glass cylindrical tank and Mass culture: In 90 letters glass aquaria.

**2.1. Culture procedure**

Indoor culture consists of maintenance and stock cultures in 250 ml flasks, starter culture in 2L plastic bottles and 10L polyethylene bags in an air-conditioned laboratory. One liter stock solution are made and used to enrich the indoor algae medium. The medium is then provided with trace metals using the Hawaiian Oceanic Institute mixture (**Millot *et al.,* 2008**). Each sterilized flask is filled with 150 ml of the sterilized prepared medium. Flasks then inoculated with algae using healthy stock or maintenance culture. Three ml of *N. oculata* is added to each 150 ml prepared medium to give an initial density of $6\text{-}8\times10^4$ cell/ml. Only 0.15ml algae are added to each 150 ml culture to start a maintenance culture. The flasks are closed using aluminum foil stopper after inoculation. The nutrient stock solutions for 1 liter distilled water were Potassium nitrate 202g; Sodium Phosphate (mono basic) 36.9g; concentrated HCl, 28ml; Calcium chloride, 50.6g; $FeCl_2$, 5g; Na EDTA, 7g; $CoCl_2$, 0.4g/25ml and $MnCl_2$, 8.5/25ml**.** The formulated trace metal mixtures were $H_3$ BO, 0.88 g; $CuSO_4$+5 $H_2O$, 0.1g;Na–EDTA,36g; $MgSO_4$+7 $H_2O$, 13.6g; $NaMoO_4$, 3.6g and $ZnSO_4$+7 $H_2$ O, 3.1g (according to **Millot *et al.,* 2008).**

Outdoor culture begins in 2-letter plastic bottle and is then up scaled to 10 liters bags which are the stock for the cylindrical fiberglass tank, the intermediate outdoor culture. To each 2 and 10 liters starter culture the sterilized prepared medium is added. Laboratory stock culture is used to inoculate the 2L bottle. One flask is needed for each bottle. The initial density is $3.2\text{-}4\times10^5$ cells/ml. To inoculate the 10L starter culture one 2L bottle is used. The initial density is $3.5\text{-}4\times10^6$ cells/ml. Intermediate cultures is conducted outdoor in a fiber glass cylindrical tank prepared to provide the glass aquaria with continuously algae flow. Nutrient mixture of inorganic fertilizers is added to the water flow to mix the sea water with fertilizers while the water flow entering the intermediate culture. Two bags of 10-letter starter culture are put in the 180L fiberglass cylindrical tank then sterilized sea water with the fertilizers then flow to the cylindrical tank from an 85L glass aquaria over it.

Mass culture is started after the intermediate culture is completed. The 85L glass aquarium over the tank is filled daily by sea water at salinity of 25‰. The sterilized sea water and the fertilizers are then introduced to the cylindrical tank. The over flow of the algae produced at the cylindrical tank is transferred to a group of glass aquaria by means of a PVC tubes branched to the aquaria each with a valve to distribute the produced algae to one of the glass aquaria to complete mass culture in.

**2.2. Culture condition:**

Flasks are kept under constant illumination using four 34-watt fluorescent lamps suspended behind the flasks. Aeration did not provide at this stage to minimize the risk of contamination. Flasks are shaken several times daily to prevent the cells clumping at the flask bottom. The peak density is $3\text{-}3.5\times10^6$ cells/ml of *Nannochloropsis oculata* could be obtained five to six days after inoculation. The culture condition of micro algae according to **Anonymous (2001)**: Temperature: ranged from 18° to 24°C; Salinity from 20 to 35 ppt; Light from 1000 to 2000lux for smaller volumes up to 10000 lux for larger tanks, where light penetration is limited; pH: normally increased up to 9 by the photosynthetic activity of the algae themselves, it is kept at a lower value (8-8.5) by the addition of acid or carbonic gas ($CO_2$).

**2.3. Rotifer (*Brachionus plicatilis*):**

The rotifer *B. plicatilis* is a rather sturdy species able to tolerate a wide range of salinity, temperature and ammonia levels. It can also use several food sources, provided that particle size remains within a 2-20 μm range. For the breeding of many marine finfish species the rotifer is, up to now, the only live food that can be used in their very early larval stages. Among other valuable characteristics as live food for fish, *B. plicatilis* was also chosen due to the relative easiness to culture it in large scale. With a proper inoculum and under optimal rearing conditions, a rotifer population should reach its harvesting density within 4 to 5 days.

Rotifer and algae are given to the larvae after attained 2-3 days old. The algae do not only serve as a food for the gilthead sea bream larvae, but also serves as a food for rotifer and helping to improve the water quality. Every day before exchanging the water the rotifer remaining in the rearing aquarium is counted in order to adjust the amount of rotifer given daily. To ensure that the availability of food is adequate for the fry during 24h period, rotifers were culture in 30 liter volume containers. Each container was fitted with one airline and air stone and an aquarium heater with thermostat. Water temperature was maintained at 22-26°C, and salinity at 30-35 ppt.

**Culturing of *Brachionus plicatilis*:**

**Semi – continuous culture using green micro algae as feed**

*Nannochloropsis oculata* is used to feed the rotifer. The cell density is $1.1\text{-}1.4\times10^7$/ml to start a new culture in 10 L aquarium, the aquarium is filled with 1L of microalgae at first time and inoculated with $5\text{-}6\times10^4$ rotifers from another aquarium, resulting in an initial density of 40-50/ml. The aquarium is topped off with 4L of green microalgae the next day. On day 2, the rotifer density increases to 90-120/ml, at which time they are ready for harvesting. About half of the rotifer, 2.500.000-2.750.000 is harvested daily. After every harvested the amount of water removed is replaced by an equal volume of microalgae; harvesting continues for about five days. Thereafter, the total crop is harvested and the aquarium is washed and prepared for a new culture. The whole cycle produce 2.500.000-2.750.000 rotifer per cycle. The total volume of microalgae used is 21.5 L per day.

**Artemia:**

Artemia is given when the larvae aged 20 days or attained the size about 5.3-5.7mm total length. The amount of brine shrimp nauplii daily given and adjusted according to the number

of nauplii remaining in the rearing aquarium in the following day. Artemia cysts were hatched at 28ºC for 18 hours. The nauplii were counted after hatching

**Hatching protocol of Artemia cyst:**

Hydrate cysts were placed for 1 h in water, with aeration at 28°C then transferred to 1.5L sea water with 6 um hydrogen peroxide in transparent bottles supplied with air through strong aeration line. Cysts were incubated at density of 2 g/l.5L for 24 hr. After hatching, all cysts in suspension separated from the hatching wastes (empty cyst shells, unhatched cysts, debris, microorganisms and hatching metabolites). Five to ten minutes after switching off the aeration, cyst shells floats and removed from the surface, while nauplii and unhatched cysts concentrate at the bottom. Then the newly hatched nauplii harvested by siphoning using a clean transparent rubber tube.

**Fatty acid enrichment:**

Newly hatched nauplii are transferred to the enrichment tank at a density of 30–50 nauplii/ml. The enrichment medium consists of 1.5ml fish oil emulsified with 50ml neutralized seawater maintained at 25$^{o}$C using 0.1gm phosphatedyl choline (Lecithin).

The enrichment emulsion is added in consecutive doses of 0.3 g/l every12 h. Strong aeration using air stones or pure oxygen is required to maintain dissolved oxygen levels above 4 ppm. Enriched nauplii are harvested after 24 h, HUFAs are obtained after 24-h enrichment with the emulsified concentrates. Nauplii should be transferred or exposed to the enrichment medium as soon as possible before first feeding, so they begin feeding immediately after the opening of the alimentary tract (instar II stage). As a result, the increase of nauplii size during enrichment can be minimized.

**Experimental design:**

Apparently healthy gilthead seabream, *(Sparus aurata*) larvae were obtained from the hatchery of the General Authority of Fish Resources Development (GAFRD) at Kilo 21 in Alexandria and transferred to the MFL aquaria. Larvae were passed through a potassium permanganate bath (20 ppm concentration) for 1 hour to prevent any infectious agent. During the adaptation period, all fry were daily fed on rotifer and Artemia. At the end of adaptation period, 525 Seabream larvae (0.5-0.7±0.01 mg in weight and 9-10±1mm for length) were divided into seven groups in three replicates for each treatment, therefore twenty one aquaria (100×30×40 cm) were required and stocked by 25 larvae for each aquarium. The aquaria were supplemented with continues aeration. Fish samples were collected biweekly for determination body weight , body length, mortality and survival rate.

Immediately prior to stoking with larvae, each aquarium was filled with 100L seawater (31-35 ppt) and 2L of *Nanochloropsis Oculata* algae as a water conditioner. The micro alga also serves as food for the rotifer *Brachionus plicatilis,* which is used as feed for larvae during the experimental period at a density of 8 rotifers per ml.

The experiment started and the larvae were weaned after 25, 27, 29, 31, 33, 35, 37 and 39 day for the different larvae groups, respectively.

Larvae samples were done biweekly using a seine net. Larvae were individually weighed (g), while total length was measured (mm) for all aquaria, artemia was gradually replaced by artificial diet within 5 days for all aquaria. Formulation and composition of the artificial diet was illustrated in Table (1).

**Diets preparation**:

The air dry ingredients (able 1) were grinded to fine granules. Ingredients were weighed and mixed mechanically by horizontal mixture at the middle speed for 10 minutes. After complete homogenization the mixture was obtained, the 40ml hot water per 100g diet was slowly added to the mixture. The mixing speed was increased for 5 minutes during water addition and at the begging to clump formulation diets were pelleted by electric meat grinder (1mm/mach). The pellets were dried in a drying oven for 24 hours on 65$^{o}$C and stored in plastic bags and finally kept at 4$^{o}$C during the experimental period to avoid rancidity.

**Growth performance parameters:**

At the end of the experiment, all fish were counted, individually weighed (g), while total length was measured (mm) from the anterior part of fish to the end of its tail. Fish growth performance was calculated using the following equations:

Weight gain = final mean weight (g) – initial mean weight (g)

Specific growth rate=(ln final weight (g) – ln initial weight(g)/time×100.

Survival rate (%)=(initial number - number of dead fry)/initial number×100

**Environmental conditions:**

During the experimental period, natural light was available providing nearly 12 hrs light/day at temperature 18-22$^{o}$C. Each aquarium was cleaned every morning prior to feeding by siphoning the wastes which had accumulated on the bottom, to protect fish in the aquarium from the adverse effect of these wastes. Moreover, the tanks were scrubbed and thoroughly cleaned at weekly intervals. Water samples were taken weekly from each aquarium for determination the physico-chemical parameters. Temperature was measured at the site of sampling, using an oxygen-meter. Dissolved oxygen was measured at the site of sampling, using an oxygen-meter. pH was measured by using glass electrode pH-meter. Salinity was measured by using a salinity-conductivity meter.

**Proximate composition of fish and experimental diets**

At the end of each experiment, two fish were randomly sampled from each aquarium and subjected to the chemical analysis of whole fish body. Moisture, dry matter (DM), ether extract (EE), crude protein (CP), crude fiber (CF) and ash content of diets and fish were determined according to the methods described in **AOAC (1990):** dry matter after drying in an oven at 105°C until constant weight; ash content by incineration in a muffle furnace at 600°C for 12 hrs; crude protein (N×6.25) by the kjeldhal method after acid digestion; and ether extract by petroleum ether (60-80°C) extraction.

**Statistical analysis:-**

Statistical analysis of the obtained data was analyzed according to **SAS (1996).** Differences between means were tested for significance according to Duncan's multiple rang test as described by **Duncan (1955).** The following model was used to analyze the obtained data: $Y_{ij} = \mu + \alpha i + e_{ij}$

Where: $Y_{ij}$ = the observation on the ij$^{th}$ fish eaten the i$^{th}$ diet; $\mu$ = overall mean, $\alpha_i$= the effect of i$^{th}$ treatment and $E_{ij}$ = random error assumed to be independently and randomly distributed $(0, \delta^2 e)$.

Table (1): Formulation and composition of the artificial diet used for seabream

| Ingredient | Quantity |
|---|---|
| Wheat mil | 20 |
| Fish meal | 30 |
| Dried milk | 10 |
| Wheat bran smooth | 13 |
| Corn gluten | 5 |
| Cocked smooth Soya | 20 |
| Vit. E | 0.3 |
| Vitamin and minerals mixture | 0.3 |
| Common salt | 0.5 |
| Carboxy methyl cellulose | 0.3 |
| Fish oil | 0.5 |
| Zymogin | 0.1 |
| ***Proximate analysis of artificial diet* (%)** | |
| Moisture | 12.16 |
| Crude protein | 44.15 |
| Ether extract | 19.18 |

| | |
|---|---|
| Crude fiber | 4.10 |
| Ash | 5.29 |
| Metabolizable energy (ME) Kcal/kg diet | 3361 |

## RESULTS AND DISCUSSION

Weaning is the gradual shift in feeding from live food onto artificial diets during the larval rearing of fish. The effect of diets (live food or artificial diets) on fish larvae growth has commonly been evaluated based on body weight, body length, weight gain and specific growth rate and these parameters can offer a full image of the physiological condition and larvae growth.

### 1. Larval weight and length:

Average larvae wet weight at the rearing day 30 dph ranged between 0.147 to 0.152 gm (table2). Larvae group weaned at 31 dph recorded the highest body weight over all group (0.152 gm) followed by the same weights for groups 33, 36 and 39 (0.150 gm), while the lowest body weight recorded for larvae group weaned at 27 dph (0.147 gm).

The mean wet weight of gilthead seabream larvae after 45 dph (Table, 2) showed that, the late weaning (33, 36 or 39 dph) significantly increased the wet weight of larvae compared to larvae groups that early weaned (25 and 27 dph) and the same trend was also observed for gilthead larvae after 60 dph.

The average larvae length at the rearing day 30 dph ranged between 7.33 to 7.57 mm for the different larvae groups. Larvae group weaned at 36 dph showed the longest larvae (7.57 mm) while the early weaned larvae (25 dph) showed the shortest larvae (7.33 mm). At the rearing day 45 dph ranged between 0.96 to 1.27 cm. At the rearing day 60 dps mean body larvae lengths ranged between 1.38 to 1.70 cm and the differences between these means were significant.

Results showed that the late weaning of gilthead seabream larvae significantly increased larvae body weight, while the early weaning significantly reduced larvae body weight and body length (Tables, 2, 3). The poor performance of artificial diets is due to low residence time in the water, low palatability, and low ingestion rates due to low digestibility of the diet because of inadequate digestive enzyme activity or poor nutritional composition of the diet compared to the natural food available to the larvae before weaning. Therefore, a microdiet formulated to early weaning of fish larvae must cover all nutrients to the larval requirements in order to improve larval nutrition, and thus enhance larval growth and survival following weaning **(Cahu, *et al.,* 1999 and 2003 and Hamza *et al.* 2008).**

It is known that the digestive enzyme system of early larvae is poorly developed and that the digestion of food is assisted by the autolysis of natural prey resulting from the enzymes contained within that prey **(Munilla-Moran *et al.,* 1990).** The formation of the stomach and the commencement of peptic enzyme production appear to be critical to successful digestion of conventional artificial diets **(Segner *et al.,* 1993).**

The obtained results in the present study are relatively in agreement with those obtained by **Ribeiro, *et al.,* (2005)**. They evaluated the weaning success of *Solea senegalensis* larvae. One group of larvae was fed only with enriched Artemia (live food only) whereas in another group, the Artemia were gradually replaced by the formulated diet over a period of 39 days. Body weight did not significantly ($P>0.05$) affected by the feed (Artemia or artificial diet) at the rearing days 0 and 9 but after this date (at the rearing days 23, 30 and 39 dph) larvae fed Artemia showed the heavies larvae ($P<0.05$) compared to that of fed the artificial feed.

In recent study, **Bonaldo *et al.,* (2011)** studied the feasibility of weaning common sole (*Solea solea* L.) larvae at 13 days post hatching (dph) using commercial microdiets (MDs)

while reducing live-feed utilization. Four dph larvae were fed two kinds of experimental weaning feeding regimens (FRs): control where larvae were weaned onto MD at 27 dph; whilst the other FRs provided live food until 13 dph. The trial lasted 29 days. At the end of the trial, live food regimens showed significantly lower weight and higher length when compared to control.

**Blair *et al.*, (2003)** Mean haddock (*Melanogrammus aeglefinus*) larval weights were significantly different ($P$<0.001) between rotifer (1.61 mg) and *Artemia* nauplii (2.14 mg) treatments. The weights of larvae fed microdiets (0.75, 0.85 and 0.86 mg) were not significantly different from each other; however, all microdiet treatments were significantly ($P$<0.01) smaller than the live food treatments.

Generally, our results indicated that, during the first three weeks of the experiment, weight and length did not increase markedly and this could be considered a long adaptation period to the new food; however, after this period the growth pattern changed radically for all treatments, particularly for *Artemia.* During the first two weeks of the experiment, the results obtained in the *Artemia* treatment were satisfactory in terms of body weight and body length considered that weaning success depends not only on the quality of the diet, but also on the physiological state, developmental stage and age of the larvae. Furthermore, the present results suggest that the first four weeks of weaning are a critical period, with a tendency towards growth diminution and depletion of the energy reserves. A depletion of the lipid stores seems particularly important when fish are weaned early.

Table (2): Effect of weaning age on weight of gilthead seabream larvae.

| Weaning age | Number of larvae | Weight 30 | Weight 45 | Weight 60 |
|---|---|---|---|---|
| | | Mean ± SE | Mean ± SE | Mean ± SE |
| 25 | 15 | 0.148 ± 0.001 c | 0.185 ± 0.002 c | 0.224 ± 0.001 d |
| 27 | 15 | 0.147 ± 0.001 c | 0.186 ± 0.002 c | 0.237 ± 0.001 c |
| 29 | 15 | 0.149 ± 0.001 bc | 0.190 ± 0.002 b | 0.241 ± 0.001 b |
| 31 | 15 | 0.152 ± 0.001 a | 0.193 ± 0.002 b | 0.242 ± 0.001 b |
| 33 | 15 | 0.150 ± 0.001 b | 0.197 ± 0.002 a | 0.247 ± 0.001 ab |
| 36 | 15 | 0.150 ± 0.001 b | 0.199 ± 0.002 a | 0.251 ± 0.001 a |
| 39 | 15 | 0.150 ± 0.001 b | 0.199 ± 0.002 a | 0.252 ± 0.001 a |

Means followed by different letters for each column are significantly different (p<0.05)

Table (3): Effect of weaning age on length of gilthead seabream larvae.

| Weaning age | Number of larvae | Length (mm) after 30 day | Length (cm) after 45 day | Length (cm) after 60 day |
|---|---|---|---|---|
| | | Mean±SE | Mean±SE | Mean±SE |
| 25 | 15 | 7.33±0.03 e | 0.96±0.02 d | 1.38±0.02 e |
| 27 | 15 | 7.41±0.03 d | 0.98±0.02 d | 1.43±0.02 d |
| 29 | 15 | 7.41±0.03 d | 1.09±0.02 c | 1.45±0.02 d |
| 31 | 15 | 7.53±0.03 ab | 1.19±0.02 c | 1.51±0.02 c |
| 33 | 15 | 7.43±0.03 cd | 1.11±0.02 b | 1.61±0.02 b |
| 36 | 15 | 7.57±0.03 a | 1.23±0.02 ab | 1.59±0.02 b |
| 39 | 15 | 7.49±0.03 bc | 1.27±0.02 a | 1.70±0.02 a |

Means followed by different letters for each column are significantly different (p<0.05)

**2. Weight gain**

As shown table 4, the average weight gain for the experimental period 30-45 dph for the different larvae groups ranged between 0.036 and 0.048 gm for the different larvae groups and ranged between 0.039 to 0.052 gm during the period 45-60 dph. The early weaning

groups (25, 27 and 29 dph) significantly decreased larvae weight gain while the late weaning (36 and 39 dph) significantly improved weight gain and the same trend was also observed during the entire experimental period (30-60 dph).

The obtained results are agreed with those obtained by **Kestemont *et al.,* (2007)** when common perch *Perca fluviatilis* larvae fed *Artemia nauplii* after hatching and then divided into different groups (12, 19 and 26 dph) receiving *Artemia nauplii* as control or artificial diet, they found that the best weight gain (380.8 mg) was obtained in larvae weaned at 19 dph, whereas the lowest weight gain (218.9 mg) was obtained in fish weaned at 12 dph.

The superiority of late weaning of seabream larvae may be due to the long period that larvae fed on the live food compared to the early weaning which depending on the formulated diets. Live food organism (Artemia) is thought to stimulate larval feeding by their movement and metabolic wastes and chemical attractants (mainly free amino acids) **(Cahu and Zambonino- Infante, 2001 and Kolkovski, 2001 and Kolkovski *et al.,* 2004**). Moreover, live food organisms are more readily digested than microdiets and contribute to the digestive process by providing exogenous enzymes **(Baskerville-Bridges and Kling, 2000**).

The free amino acids alanine, glycine and arginine and the compound betaine were identified from the *Artemia* rearing medium as metabolites stimulate gilthead seabream, *Sparus aurata* larvae response. Live food may also contribute exogenous enzymes to the digestion process or provide factors that stimulate larval pancreatic secretions or activate gut zymogens (**Koven *et al.,* 2001**). The same authors indicated that, factors in live *Artemia* may influence digestion by stimulating an endocrine response. This was shown when *Artemia* consumed by seabream larvae elicited a 300% increase in the level of the digestive hormone bombesin compared to levels in larvae given only a microdiets.

During the past three decades, enormous efforts have been made to develop microdiets (MD) to replace live food, both rotifers and *Artemia,* as complete or partial replacements for marine fish larvae. However, although, there were substantial achievements in reducing the reliance on live foods and weaning the larvae earlier onto microdiet still in most species, microdiets cannot completely replace live foods.

There are several reasons why microdiets cannot, at this stage, completely replace live food. Nutritional profile for marine fish larvae is not yet to be completely defined. Although lipids and fatty acid requirements are known (to a degree), very little work was carried out to define the protein / amino acids, mineral and vitamins. Chemical and physical properties are playing a crucial role with the behaviour of MD particle in the water, attractability, leaching, ingestion and ingestion in the larvae gut. However, very little attention was giving to these issues.

The relatively low level of enzyme activities and the absence of pepsin-like enzyme activity has been considered as one of the reasons for the limited success of microdiets and the poor growth of larvae fed solely on formulated diets (**Kolkovski et *al.,* 1997a and Day *et al.,* 1997**).

Table (4): Effect of weaning age on weight gain (gm) of gilthead seabream larvae.

| Weaning age | Number of larvae | WG 30-45 | WG 45-60 | WG 30-60 |
|---|---|---|---|---|
| | | Mean ± SE | Mean ± SE | Mean ± SE |
| 25 | 15 | 0.036±0.0003 d | 0.039±0.0004 b | 0.076±0.0003 c |
| 27 | 15 | 0.039±0.0003 c | 0.051±0.0004 a | 0.090±0.0003 b |
| 29 | 15 | 0.040±0.0003 b | 0.051±0.0004 a | 0.092±0.0003 b |
| 31 | 15 | 0.041±0.0003 b | 0.049±0.0004 a | 0.089±0.0003 b |
| 33 | 15 | 0.046±0.0003 a | 0.050±0.0004 a | 0.096±0.0003 ab |
| 36 | 15 | 0.048±0.0003 a | 0.052±0.0004 a | 0.100±0.0003 a |
| 39 | 15 | 0.048±0.0003 a | 0.053±0.0004 a | 0.101±0.0003 a |

Means followed by different letters for each column are significantly different ($p<0.05$)

The lack of suitable microdiet that can replace live food and be manipulated to address the nutritional requirements of fish larvae is still the bottle neck in marine fish larvae nutritional studies. An integrative approach needs to be taken in the development of microdiets for fish larvae taking into the larval physiology; digestive system, and nutritional requirements (lipids, proteins, vitamins and trace elements) as well as technology (leaching, sinking, binders, feeding and rearing systems). Microdiet manufacturers need to focus on better ingestion, digestion and assimilation of a balanced nutrient profile that is provided using an all-encompassing approach. To date, larval nutritional requirements are only partially identified and much is still unknown.

**Specific growth rate (SGR) of gilthead seabream larvae:**

During the entire experimental period the highest SGR values were recorded for groups 39 and 36 (1.71 and 1.70), while the lowest SGR recorded for group 25 (1.39) indicating that, the late weaning age released the best SGR compared to the early weaning of gilthead seabream larvae. The early weaning of gilthead seabream (*Sparus aurata*) larvae significantly reduced larval growth than larvae fed the live food. Low ingestion rates of the artificial diet combined with a reduced ration of Artemia during the weaning period is probably the main reason why reduced growth for the early weaned larvae.

The obtained results in our study are in agreement with those obtained by **Guerreiro (2010)**. He indicated that Early-weaned White seabream (*Diplodus sargus*) larvae (20 dph) exhibited a lower growth compared with normally weaned larvae (27 dph) but the pattern of Relative Growth Rate (RGR) variation was identical for both feeding regimes. The study suggests that an inert diet can be included in the feeding regime of white seabream as early as 20 dph. **Aquacop and Nedelec (1989)** reported that, the weaning of seabass larvae on artificial pellet is feasible as early as the 25$^{th}$ day when the mean weight of fry ranges from 20 to 40 mg with alternative food sequences of live to frozen Artemia and dry pellet containing a high protein (56 %) and lipid (16 %) level. **Kestemont *et al.* (2007)** indicated that the best SGR of pikeperch larvae *(Sander lucioperca)* was found when co-feeding was started at 19 dph compared to 12 dph or 26 dph.

Complete elimination of *Artemia* by weaning of fish larvae onto microdiets directly from rotifers with acceptable survival and/or growth rate has been reported for red drum *Sciaenops ocellatus* (**Holt 1993**), cod, *Gadus morhua* **(Rosenlund and Halldorsson 2007 and Wold, *et al.*, 2007)** and Asia sea bass *Lates calcarifer* **(Curnow, *et al.*, 2006 a& b).**

Table (5): Effect of weaning age on specific growth rate of gilthead seabream larvae.

| Weaning age | No. of larvae | Mean ± SE |
|---|---|---|
| 25 | 15 | 1.39 ± 0.01 c |
| 27 | 15 | 1.60 ± 0.01 b |
| 29 | 15 | 1.59 ± 0.01 b |
| 31 | 15 | 1.54 ± 0.01 b |
| 33 | 15 | 1.65 ± 0.01 ab |
| 36 | 15 | 1.70 ± 0.01 a |
| 39 | 15 | 1.71 ± 0.01 a |

Means followed by different letters for each column are significantly different ($p<0.05$)

Although weaning can be achieved at metamorphosis or 0.50-0.75 g in most species (**Hardy, 1989**), the earlier introduction of formulated diets as the sole replacement for live food had only limited success (**Walford *et al.*, 1991; Kolkovski, 1995 and Kolkovski and Tandler, 1995**). The poor performance of formulated diets is related to the inadequate incorporation of nutrients due to poor ingestion, digestion and/or assimilation (**Kolkovski and Tandler, 1995 and Kolkovski and Dabrowski, 1999**).

Recent successes with replacing or reducing live-feed dependence were achieved by co-feeding of live and dry feeds. Weaning to dry diets is done gradually, starting with co-feeding and reducing the amount of live foods (usually *Artemia*) until replacement with dry diets is completed (**Kolkovski *et al., 1*997 a).**

Feeding prey organisms, such as *Artemia* nauplii, a short time before dry diets are offered may increase digestive activity and increase the ability of larvae to digest the dry diets **(Kolkovski *et al.,* 1997 a)**. Many works have described the advantages of co-feeding dry and live food to fish larvae (**Person-Le Ruyet *et al.,* 1993; Walford and Lam, 1993; Lavens *et al.,* 1995 and Rosenlund *et al.,* 1997**). For example, **Tandler and Kolkovski (1991)** achieved 80% survival with no growth problems using the co-feeding technique for rearing 10-day-old gilthead seabream larvae. **Kolkovski *et al.,* (1997 b)** reported a significant increase in formulated diets assimilation when seabass larvae were co-fed formulated diet and *Artemia* nauplii compared with larvae fed solely on formulated diet. One possible explanation for improved larval growth and increased nutrient assimilation when co-fed live and dry diets, may be related to the contribution of digestive enzymes from the live food organisms. Some authors have suggested that larvae utilize enzymes from their prey to facilitate the process of digestion until the larval alimentary system is fully differentiated and developed (**Kolkovski *et al.,* 1993**). This hypothesis was supported by **Pedersen and Hjelmeland (1988)** who showed that, in addition to the exogenous proteolytic enzyme activity, live prey induce an increase in endogenous trypsin secretion in herring *Clupea harengus* larvae. Alternatively, the products of live prey autolysis, possibly including neurohormonal factors **(Chan and Hale 1992),** may stimulate secretions of trypsinogen from the pancreas and/or activate gut zymogens (**Person-Le Ruyet *et al.,* 1993**).

It is clear that live food organisms contribute to the digestion and assimilation process in fish larvae. However, their contribution may be in forms other than direct enzymes contribution. For example, **Kolkovski *et al.* (1997 a)** and **Koven *et al.,* (1998)** demonstrated that lipid fractions such as phosphatidylcholine and lysophosphatidylcholine, extracted from *Artemia* lipids, enhance assimilation of formulated diets in gilthead seabream larvae. Other protein and/or lipid fractions may be involved directly or indirectly as precursors or activators in the assimilation and absorption process.

In the elaboration of feeding strategies to obtain optimal growth in the larviculture of marine species, it is essential to consider the fish capacity for food digestion during larval development. There are differences in the ontogeny of the digestive tract among fish species, but most marine fish species lack a functional stomach during the larval phase until metamorphosis (**Garcia-Ortega, 2009**).

On day 5 post hatching about 50% of the gilthead seabream (*Sparus aurata*) larvae had a full stomach. A rotifer population of 2-3/ml was maintained in the tank. On day 10, 75% of the larvae were found to have developed the swim bladder. On day 22 freshly hatched Artemia were given, gradually decreasing the quantity of rotifers. From day 28 the larvae were fed with *Artemia* nauplii enriched with HUFA. Most hatcheries wean gilthead seabream (*Sparus aurata*) when they are 40-55 dph **(Cataudella *et al.,* 1995).**

It seems that the ability to utilize microdiets of gilthead seabream (*Sparus aurata)* dependent on larval development of digestive system and diet attractiveness. Weaning of Asian sea bass larvae onto a commercial microdiet with better attractive properties (higher content of free amino acid and lipid) resulted in higher larval growth rate **(Curnow *et al.,* 2006 b)**. The artificial diet used in our experiment seemed to not cover all gilthead seabream (*Sparus aurata*) requirement with low attractive properties suggesting that this microdiet is not the optimal for early weaning of gilthead seabream larvae.

**Survival rate of gilthead seabream:**

After 30 days from hatching, survival rates for seabream larvae groups ranged from 78.67 to 88.00 for the different larvae groups and ranged between 88.00 to 99.00 at 60 dph (Table, 6) and the differences between these averages were significant.

The obtained results indicated that, the early weaning (25 dph) for gilthead seabream larvae significantly reduced survival rate compared to late weaning (36 and 39 dph).

The highest mortality rates at the early weaning ages (25 dph) could be the results of problems such as feed competition or failure in ingestion the artificial diets or live preys during the first feeding days **(Benetti *et al.*, 2008)**. Disinfection of eggs and rearing water and application of probiotics in artificial diet were likely the key factors to minimize mortality in gilthead seabream larvae. These practices were not applied in our experiment, but could be useful in our future weaning studies. In addition, using a larger tank volume than in our experiments with a lower larval stocking density may provide better hydro-dynamic condition and keep the water environment (i.e. temperature) more stable, probably also improves the larval survival.

The obtained results agreed with those obtained by **Kestemont *et al.*, (2007)** when common perch *Perca fluviatilis* larvae fed with *Artemia nauplii* after hatching and then divided into different groups (12, 19 and 26 dph) receiving *Artemia nauplii* as control or artificial diet, they found that, the lowest mortality (48.1%) was obtained in larvae weaned at day 19 post-hatch, whereas the highest mortality (68.6%) was obtained in fish weaned at day 12 post-hatch.

Table (6): Effect of weaning age on survival rate of seabream larvae.

| Day post hatching (dph) | Survival rate % (30 dph) | Survival rate %(60 dph) |
|---|---|---|
| 25 | 78.67 ± 2.67 b | 88.00 ± 1.00 b |
| 27 | 84.00 ± 2.31 a | 90.33 ± 2.60 b |
| 29 | 86.67 ± 1.33 a | 89.00 ± 3.00 b |
| 31 | 88.00 ± 2.31 a | 86.33 ± 6.33 b |
| 33 | 85.33 ± 1.33 a | 92.00 ± 4.16 b |
| 36 | 88.00 ± 2.31 a | 99.00±3.10 a |
| 39 | 88.00 ± 2.31 a | 89.33 ± 3.48 b |

Means followed by different letters for each column are significantly different ($p<0.05$)

**Ribeiro, *et al.*, (2005)** found that**,** Sengegalese sole, *Solea senegalensis* larvae fed the *Artemia* diet exhibited higher survival rates than the fish fed the artificial diet ($P<0.05$). **Blair *et al.*, (2003)** found that, survival of haddock (*Melanogrammus aeglefinus*) significantly higher ($P<0.001$) for larvae fed rotifer and *Artemia nauplii* than larvae fed the microdiet. **Nguyen *et al.*, (2011)** showed that, weaning of cobia *(Rachycentron canadum* L.) larvae onto a microdiet directly from rotifers significantly reduced survival rate of the larvae and did not lead to larval acceptance of the microdiet, compared to those weaned from *Artemia* in the first experiment. Early weaning of cobia larvae onto microdiet (on 8 or 13 dph) from *Artemia* in the second experiment also reduced survival rate and gut maturation index, compared to those fed live food.

In conclusion, early weaning of gilthead seabream (*Sparus aurata*) larvae gradually onto a microdiet from Artemia significantly reduced growth and survival rate of the larvae, and did not lead to good larval acceptance of the microdiet, compared to those weaned from *Artemia.*

Results of growth and survival rate in the present study indicated that, BW, BL, GW and SGR did not significantly affected when larvae weaned at 31, 33 of 36 dph and this agreed to the results of **Cataudella *et al.*, (1995).** The obtained results indicated the possibility of early weaning of seabream larvae at 31dph without significant effect on growth parameters (BW, BL, WG and SGR, tables, 2, 3, 4 and 5) and this early weaning protocol allows a reduction of 24 day in the use of live food in feeding protocol for seabream larvae as recommended by **Cataudella *et al.*, (1995).** Thus improving the cost-effectiveness of seabream fish larvae production.

However, growth improvement of juveniles in the early co-feeding treatments 31, 33 and 36 was small. This result indicated that the present nutritional composition of prepared diet used in the present study is still not optimal for sea bream larvae during the early co-feeding period. Therefore, more research on the larval nutritional requirements needs to be conducted and the appropriate nutritional composition of formulated diets for seabream larvae needs to be developed.

**larval protein and fat content of gilthead seabream**:

Protein and fat content of gilthead seabream *(Sparus aurata)* larvae at the end of the experiment ranged between 31.20-36.20 for protein and 2.10-3.36% for fat, respectively (Table 7). The obtained results indicated that the late weaning of larvae (36 and 39 dph) significantly increased each of protein and fat content of larvae when compared to the early weaning of larvae (25 and 27 dph). For the same trend, **Ribeiro, *et al.,* (2005)** found that, Senegalese sole *Solea senegalensis* postlarvae fed a compound diet adapted to the diet at the end of the experiment, but postlarvae from the *Artemia* treatment exhibited significantly higher values for protein and total lipid contents.

A significant effect of the artificial diet was observed on the postlarvae body composition. During the early weaning of larvae by gradually increasing the artificial diet and decreasing live food significantly decreased specific growth rate after the concomitant increase of the artificial diet in the daily ration and live food was insufficient to fulfill postlarvae energetic requirements, leading to a decrease in growth, the body content of protein and fat while the late weaning supplied larvae by adequate amount of nutrient required for the normal growth and gained the higher amount of protein and fat in their bodies. **Hung *et al.,* (1997)** indicated that the biological and environmental factors influence the pattern of nutrient depletion in body composition.

Table (7): Effect of weaning age on protein and fat percentage of gilthead seabream larvae

| Group | Number of fish | Protein | Fat |
|---|---|---|---|
| | | Mean ± SE | Mean ± SE |
| 25 | 6 | 31.20 ± 0.66 c | 2.10 ± 0.17 c |
| 27 | 6 | 31.40 ± 0.68 c | 2.48 ± 0.08 b |
| 29 | 6 | 32.80 ± 0.97 bc | 2.80 ± 0.09 b |
| 31 | 6 | 34.20 ± 0.58 ab | 3.18 ± 0.09 a |
| 33 | 6 | 34.40 ± 0.60 ab | 3.26 ± 0.10 a |
| 36 | 6 | 35.00 ± 0.32 a | 3.24 ± 0.07 a |
| 39 | 6 | 36.20 ± 0.58 a | 3.36 ± 0.18 a |

Means followed by different letters for each column are significantly different ($p<0.05$)

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