

EFFECTS OF REPLACEMENT OF LIVE FOOD WITH DRY DIET ON GROWTH AND SURVIVAL RATE FOR SEABREAM (*SPARUS AURATA*) LARVAE

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SUMMARY

The present study was carried out to study the possibility of early weaning of gilthead sea bream (*Sparus aurata*) larvae. Therefore seabream larvae divided into different groups and weaned at 35, 40, 45, 50, 55 and 60 days post hatching (dph). At the end of the experiment the mean wet weight and length of gilthead seabream were recorded for larvae. Showed that, the late weaning (50, 55 or 60 dph) significantly increased the wet weight and length of larvae compared to larvae groups that early weaned (35 and 40 dph) and the same trend was also observed for larvae after 60 dph. During the entire experimental period the highest SGR values were recorded for groups 60 and 55 dph (3.75 and 3.19), while the lowest SGR recorded for group 35 dph (1.59) indicating that, the late weaning age released the best SGR was compared to the early weaning of gilthead seabream larvae. The obtained results indicated that the late weaning of larvae (60 dph) significantly increased each of protein and fat content of larvae when compared to the early weaning of larvae (35 dph). In conclusion, it can be concluded that, the early weaning could not only improve the growth performance, but also keep the survival rate within the same limits for the original late weaning age.

Keywords: *Live food, dry food, seabream larvae, growth performance.*

INTRODUCTION

Gilthead seabream, *Sparus aurata*, is a species of great commercial value, and an important resource of marine aquaculture in southern Europe. The major reason for the great success in the production of this species lies in its good adaptability to rearing conditions and high growth rates (Mazzola and Ralo 1981). To ensure a good growth and a high survival rate, a reliable diet that satisfies the nutritional requirements of larvae, both qualitatively and quantitatively, is essential (Kolkovski *et al.* 1993 and Sargent *et al.* 1997). 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).

Live food influences digestion by stimulating endocrine responses (Koven *et al.*, 2001). 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 *Cynoglossus semilaevis* (Chang *et al.*, 2006), striped bass *Morone saxatilis* (Chu and Ozkizilcik, 1999), barramundi *L. calcarifer* (Curnow *et al.*, 2006 a), turbot *Scophthalmus maximus* 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).

The aim of this study was to investigate the effects of early weaning strategy by graded substitute live food (*Artemia*) by artificial diets on larval growth performance and survival as criteria. Therefore, The present study investigated the effect of weaning of seabream larvae (*Sparus aurata*) at different ages 35, 40, 45 50, 55 and 60 dph on growth, survival rate and the best feeding regime required for obtaining a good quality fish larvae of Seabream under the Egyptian conditions

MATERIALS AND METHODS

Rearing system:

The larval rearing trials were carried out at Haraz Fish Hatchery - Ismailia Governorate. The water used for live food production and other activities at Haraz Fish Hatchery is transferred from the Suez canal and groundwater to the Hatchery. The water was pumped to 18 tanks (400 L for each) over the Lab. The experiment started at 2/1/2017 to 2/3/2017.

The rearing systems comprised of 400-l cylinder-conical tanks grouped in pairs. Each pair of tanks was connected to a biofilter where water was filtered mechanically. The three phases of water sterilization and treatment with U.V are used in water purification and treatment to make the water healthier and make it free of living microorganisms. The system were filled with natural seawater of 40‰ salinity and remained closed with a daily renewal rate of ~ 100-200 % until the end of the larval rearing. Aeration was provided in the tanks through a wooden diffuser during the autotrophic stage and was minimized or even stopped after first feeding and until the end of the experiment. A cooling system helped to maintain the temperature in the tanks at $19 \pm 1^{\circ}\text{C}$. Light was provided in each tank with a lamp with an intensity of 500 lux at the surface of the water. The photoperiod was established at 24 h Light – 0 h Dark since the first feeding onwards

Experimental design:

Apparently healthy gilthead seabream, (*Sparus aurata*) larvae were obtained from the hatchery (Haraz Fish Hatchery) Ismailia- Egypt. A number of 180000 Larvae were stocked in 18 tanks 400L seawater (40 ppt). Seabream larvae (0.5 ± 0.01 mg in weight and 9 ± 1 mm for length) were divided into six groups in three replicates for each treatment; the larvae were weaned after 35, 40, 45, 50, 55 and 60 day for the different larvae groups, respectively (Fig 1). Fish samples were collected weekly for determination body weight, body length, and mortality and survival rate. Immediately prior to stoking with larvae, samples were done using a seine net. Larvae were individually weighed (mg), while total length was measured (mm) for all tanks; artemia was completely replaced by artificial diet for each treatment.

Diets preparation:

Formulation and composition of the artificial diet was illustrated in Table (1). The feeding protocol was performed based on the manual of FAO (Moretti *et al.*, 1999) for 60 days. Larvae were fed 5 times daily (from 8 AM to 4 PM, one meal every two hours). The feeding rates were adjusted according to fish live body weights every 7 days.

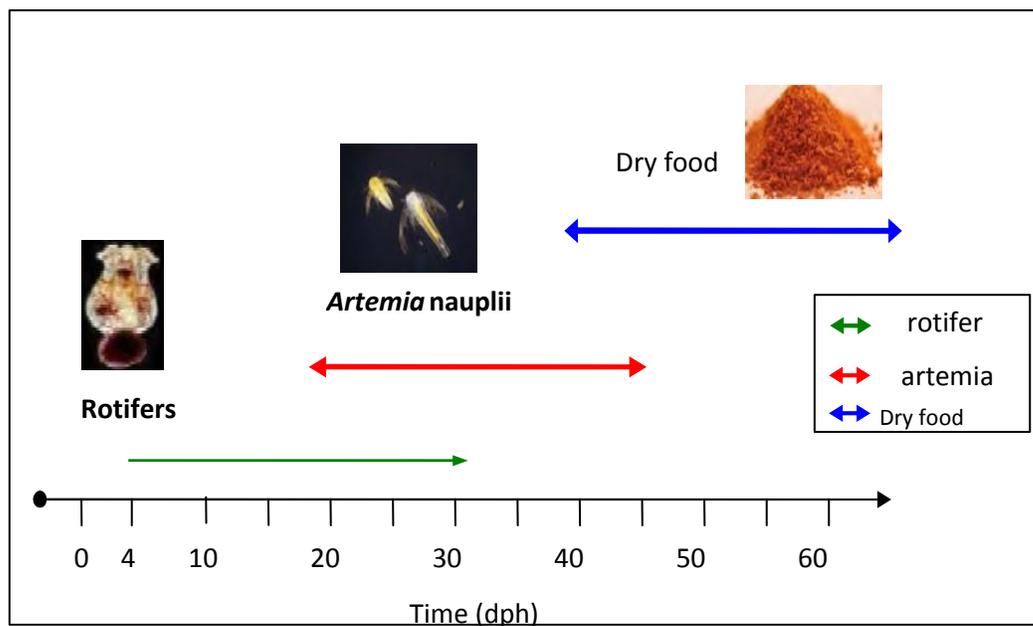


Fig 1: Larval feeding sequence for the different treatments

Table (1): Composition and proximate analysis of the experimental diets.

Ingredients	(%)
Fishmeal (70 % CP)	67
Soybean meal (44 % CP)	18
Yellow Corn	3
Fish oil	5
Sunflower oil	5
Minerals mix ¹	1
Vitamins mix ²	1
Total	100
Proximate analysis (%)	
Moisture	9.10
Crude protein	55.10
Ether extract	15.50
Crude fibre	2.00
Ash	11.90
Nitrogen free extract	6.40
Metabolizable energy (KCal / 100g) ³	396.61
Protein / energy ratio (mg P/ K Cal)	138.92

¹ and ²: NRC, 2011

³-Metabolizable energy (ME): calculated using values of 4.50, 8.15 and 3.49 Kcal for protein, fat and carbohydrate, respectively.

Growth performance parameters:

Samples for total length and wet weight were taken during the first 60 days post hatching to identify potential differences on the growth between treatments. For the TL, 20 fishes per tank every week were measured under a stereo microscope Leica type MZ 125 or Olympus Optical co., Ltd., type SZH-ILLK. . 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 (mg) – initial mean weight (mg)

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

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

Proximate composition of fish and experimental diets:

At the end of the experiment, twenty 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).

Environmental conditions:

During the experimental period, light was available providing nearly 16 hrs light/day and temperature was maintained at 19°C by a 250 watt immersion heater with thermostat. Each Tank was cleaned every morning. Water samples were taken every two days from each tank for determination the physico-chemical parameters. Where the average range of dissolved oxygen was above 5.47 mg/l. Other water quality parameter including pH was measured every two days where the average range of pH was in 7.7 ± 0.7 throughout the experiment.

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)

RESULTS AND DISCUSSION

Water Quality:

Data of water quality parameters were monitored daily and the results were not significant ($p < 0.05$) differences between treatments. The recorded values of temperature (19 °C), pH (7.60), salinity (40 ppt), and ammonia (0.02mg/L) were within the acceptable range of seabream hatcheries (Killen, *et al.* 2007; Munday, *et al.* 2009, Eldahar *et al.*, 2016).

Effect of weaning age on survival rate of seabream larval:

The survival rate of the fish larvae at the end of this experiment is shown in Table (2). The average survival rate ranged between 50 to 90 % after 53 dph and 60-90% after 60 dph. The results clearly showed that, there was a significantly difference ($P < 0.05$) between the treatments. The results of this experiment agree with the findings of larvae survival which is the most important parameter to justify the success of weaning. Increased survival with weaning age of seabream larvae as observed in the present study. The obtained results indicated that, the early weaning (35 dph) for gilthead sea bream larvae significantly ($P < 0.05$) reduced survival rate compared to late weaning (60 dph).The highest mortality rates at the early weaning ages (35 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). The results of this experiment agree with the findings of Suzer, *et al.* (2007) Suzer, *et al.* (2013).

Table (2): Effect of weaning age on growth performance parameters and survival of gilthead seabream larvae.

Parameters	Day post hatching (dph)					
	35	40	45	50	55	60
Survival rate% after (53 dph)	50.00± 2.30 ^d	65.00± 2.30 ^c	85.00± 2.30 ^b	88.00± 2.20 ^b	90.00± 2.10 ^a	90.00± 2.10 ^a
Survival rate % after(60 dph)	60.00± 1.30 ^d	70.00± 2.30 ^c	72.00± 2.30 ^c	82.00± 2.30 ^b	92.00± 2.30 ^a	92.00± 2.30 ^a
Weight mg at 53day Mean ± SE	170 ± 0.002 ^c	180 ± 0.002 ^b	0.200 ± 0.002 ^a	200 ± 0.002 ^a	200 ± 0.002 ^a	200 ± 0.002 ^a
Weight mg at 60 day Mean ± SE	190 ± 0.001 ^f	200 ± 0.001 ^e	0.230 ± 0.001 ^d	0.240 ± 0.001 ^c	250 ± 0.001 ^b	260 ± 0.001 ^a
Weight gain gm	20± 0.001 ^e	20 ± 0.001 ^e	30 ± 0.001 ^d	40 ± 0.001 ^c	50 ± 0.001 ^b	60 ± 0.001 ^a
SGR	1.59 ± 0. 01 ^e	1.51± 0. 01 ^f	2.00 ± 0. 01 ^d	2.60 ± 0. 01 ^c	3.19 ± 0. 01 ^b	3.75 ± 0. 01 ^a
Length mm at 53dayMean ± SE	12.00± 0.00 ^d	13.00± 0.002 ^c	14.00± 0.002 ^b	17.00± 0.002 ^a	17.00± 0.002 ^a	17.00± 0.002 ^a
Length mm at 60dayMean ± SE	15.00± 0.002 ^f	17.00± 0.002 ^e	18.00± 0.002 ^d	22.00± 0.002 ^c	23.00 ± 0.002 ^b	24.00± 0.002 ^a
Length gain mm	3.000± 0.002 ^e	4.00± 0.002 ^d	4.00± 0.002 ^d	5.00± 0.002 ^c	6.00± 0.002 ^b	7.00± 0.002 ^a
SGR	3.19± 0. 01 ^f	3.83± 0. 01 ^d	3.59. ± 0. 01 ^e	3.68± 0. 01 ^c	4.32± 0.01 ^b	4.92± 0.01 ^a

Means followed by different letters for each column are significantly different ($p < 0.05$) and Asia sea bass *Lates calcariferous* (Curnow, et al., 2006 a & b) and seabream (Soltan et al., 2014).

Larval weight and weight gain:

Average larvae wet weight at the rearing day 60 dph ranged between 17 to 20 mg (Table 2) The mean wet weight of gilthead seabream larvae after 60 dph showed that, the late weaning (60 dph) significantly ($P < 0.05$) increased the wet weight of larvae compared to larvae groups that early weaned (35 dph). As shown in Table (2) the average weight gain for the experimental period 53-60 dph for the different larvae groups ranged between 2 and 6 mg for the different larvae groups. The early weaning groups (35 dph) significantly ($P < 0.05$) decreased larvae weight gain while the late weaning (60 dph) significantly improved weight gain and the same trend was also observed during the entire experimental period (53-60 dph). 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. (Kestemont et al., 2007, Hamza et al. 2008 and Soltan et al., 2014).

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 (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).

Specific growth rate (SGR) of gilthead seabream larvae:

During the entire experimental period the highest SGR values were recorded for groups 60 and 55 dph (3.75 and 3.19), while the lowest SGR one was recorded for group 35 dph (1.59) 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.. 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. And also, In agreement with Soltan et al. (2014) and Eldahar et al., (2016). The study suggests that an inert diet can be included in the feeding regime of white seabream as early as 40 dph. Cod, *Gadus morhua* (Rosenlund and Halldorsson 2007 and Wold et al., 2007).

Larval length and length gain:

The average larvae length at the 53 dph weaning day ranged between 12.00 to 17.00 mm for the different larvae groups (Table 2). Larvae group weaned at 60 dph showed the longest larvae (17 mm) while the early weaned larvae (35 dph) showed the shortest length. At the rearing day 65 dph mean body larvae lengths

ranged between 15.00 to 24.00 mm and the differences between these means were significant ($P < 0.05$). 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. (Hamza *et al.* 2008). 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 53 days. Body weight was 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 53, 59 and 61 dph) larvae fed Artemia showed the heaviest larvae ($P < 0.05$) compared to that fed the artificial feed. Bonaldo *et al.* (2011)

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

The average larvae length at the weaning day 53 dph ranged between 13.00 to 17.00 mm for the different larvae groups (Table 2). During the entire experimental period the highest SGR values were recorded for groups 55 and 60 (4.32 and 4.92), while the lowest SGR recorded for group 35 (3.19) indicating that, the late weaning age released the best SGR of length compared to the early weaning of gilthead seabream larvae. The early weaning of gilthead seabream (*Sparus aurata*) larvae significantly reduced larval growth in length than larvae fed the live food. In agreement with (Soltan *et al.*, 2014). The present results are agreed with those obtained by Kestemont *et al.*, (2007)

Protein and fat content of gilthead seabream larval:

Protein and fat content of gilthead seabream (*Sparus aurata*) larvae at the end of the experiment ranged between 32.80-36.20 for protein and 2.80-3.36% for fat, (Table 3). The obtained results indicated that the late weaning of larvae (60 dph) significantly ($P < 0.05$) increased each of protein and fat content of larvae when compared to the early weaning (35 dph). For the same trend, Ribeiro, *et al.* (2005) found that, Senegalese sole *Solea senegalensis* post larvae fed a compound diet adapted to the diet at the end of the experiment, but post larvae from the Artemia treatment exhibited significantly ($P < 0.05$) higher values for protein and total lipid contents.

Table (3): Effect of weaning age on protein and fat content of gilthead seabream larvae.

Group	Number of fish	Protein Mean \pm SE	Fat Mean \pm SE
35	20	32.80 \pm 0.57 ^c	2.70 \pm 0.09 ^b
40	20	32.80 \pm 0.57 ^c	2.80 \pm 0.09 ^b
45	20	34.20 \pm 0.58 ^{ab}	3.18 \pm 0.09 ^a
50	20	34.40 \pm 0.60 ^{ab}	3.26 \pm 0.10 ^a
55	20	35.00 \pm 0.32 ^a	3.24 \pm 0.07 ^a
60	20	36.20 \pm 0.58 ^a	3.36 \pm 0.18 ^a

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

CONCLUSION

It can be concluded that, the early weaning could not only improve the growth performance, and will keep the survival rate within the same limits for the original late weaning age.

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تأثير إستبدال الغذاء الطبيعي بالعلف الجاف على معدلات النمو والإعاشة في الإنتاج المكثف ليرقات أسماك الدنيس

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³ قسم الانتاج الغذائى - معهد بحوث الانتاج الحيوانى- مركز البحوث الزراعية - مصر

⁴ قسم تغذية الاسماك - المعمل المركز بلبحوث الاحياء المائية-العباسية - محافظة الشرقية- مصر

أجريت هذه الدراسة بمفرخ حراز لتفريخ الأسماك البحرية- بمحافظة الإسماعيلية. بدأت التجربة في 2017/1/2 إلى 2017/3/2. لدراسة إمكانية الفطام المبكر ليرقات الدنيس. وتأثير إستبدال الغذاء الحي مع العلف الجاف على معدلات النمو والإعاشة في الإنتاج المكثف ليرقات أسماك الدنيس. مصدر المياه المستخدمة لإنتاج الغذاء الحي والأنشطة الأخرى في مفرخ حراز البحرى من قناة السويس والمياه الجوفية. تم ترسيب المياه قبل استخدامها وتصفيتها بفلتر ميكانيكى. كما تم تعقيم ومعالجة المياه بالأشعة فوق البنفسجية (UV) لجعل المياه أكثر جودة وخالية من الكائنات الحية الدقيقة. الظروف البيئية خلال الفترة التجريبية تحت الظروف المصرية. وقد تم تنظيم فترات الإضاءة لتصبح 16 ساعة إضاءة و 8 ساعات إظلام. وكانت قوة الإضاءة 500 لوكس. سجلت القياسات الفيزيوكيميائية للمياه المستخدمة وكانت كالاتى: درجة الحرارة 19±1 م، الأكسجين الذائب 5,5 ملجم / لتر، الملوحة 40 جزء فى الألف، الأمونيا 0,002 ملجم / لتر، والأس الهيدروجينى 7,6 ± 0,7. ومعدل تغيير المياه بنسبة 200 % يوميا حتى نهاية تربية اليرقات. تم تخزين 180000 يرقة دنيس في 18 تلك سعة 400 لتر. تم تقسيم يرقات الدنيس إلى ست مجموعات في ثلاث مكررات. معدل التخزين 10000 يرقة لكل تلك.

بدأت التجربة وتم فطم اليرقات عند عمر 35، 40، 45، 50، 55 و 60 يوماً بعد الفقس لمجموعات اليرقات المختلفة، على التوالي. تم جمع عينات يرقات الدنيس عند عمر 53 و 60 يوم من الفقس لتحديد وزن الجسم، وطول الجسم، ومعدل الوفيات ومعدل البقاء. تم وزن اليرقات بشكل فردي (مليجرام)، بينما تم قياس الطول الكلي (مليمتراً). واستبدلت تماماً الأرتيميا بالعلف الصناعى لكل معاملة فى تغذية يرقات اسماك الدنيس عند عمر 35، 40، 45، 50، 55 و 60 يوماً بعد الفقس. وكان عدد مرات التغذية 5 مرات يوميا. النتائج المتحصل عليها من التجربة:

1. سجلت أفضل زيادة معنوية فى الوزن النهائى، الطول النهائى، معدل النمو النوعى، نسبة الإعاشة كان عند المعاملة 60 يوم يليها 55، 50، يوم بعد الفقس.
 2. سجلت أقل زيادة معنوية فى الوزن النهائى، الطول النهائى، معدل النمو النوعى، نسبة الإعاشة كان عند المعاملة 35 يوم يليها 40، 45، يوم بعد الفقس.
 3. اظهرت النتائج انه يوجد اختلافات معنوية فى نسبة البروتين والدهون الخام لجسم يرقات الدنيس للمجموعات التى تم فطامها عند عمر 55، 60 يوم بعد الفقس عن باقى المعاملات.
- يستنتج من هذه الدراسة أن الفطام المبكر يؤثر سلبا على معدل الزيادة فى الوزن والطول ونسبة الإعاشة وأن الفطام عند 60 يوم بعد الفقس هو الأنسب تحت هذه الظروف التجريبية.