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EFFECT OF STOCKING DENSITY, PHOTOPERIOD AND FEEDING ON POULTRY EGG ON GROWTH AND DEVELOPMENT OF THE EGYPTIAN SOLE (*SOLEA AEGYPTIACA* CHABANAUD, 1927) LARVAE.

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Abstract

This study was conducted to evaluate the optimum stocking density, photoperiod and feeding on poultry egg on growth and development of *Solea aegyptiaca* larvae. The first experiment was tested four different stocking densities (50, 80, 110 and 140 larvae/ liter). The results found that, no significant difference in growth performance. Stocking density of 110 larvae/liter led to relatively higher survival rate compared to the other stocking densities. The second experiment was tested four different photoperiods (light, L: dark, D): 6L: 18D, 12L: 12D, 18L:6D and 24L:0D). The results indicated that, the best growth performance of larvae was at photoperiods 18L:6D and 24L:0D. Larvae exposed to photoperiods (12L:12D, 18L:6D and 24L:0D) led to relatively higher survival rate compared to 6L:18D. The third experiment was tested five different feeds type (live food (Rotifer and *Artemia*) and poultry egg (whole egg, egg white, egg yolk and boiled egg yolk)). The results indicated that, larvae that fed on live food (Rotifer and *Artemia*) showed statistically higher growth rate compared to all the other groups. It had showed the slow growth of larvae that were fed on poultry egg. Survival rate values were relatively high with fed on egg white and fed on live food.

Keywords: Stocking density, photoperiod, poultry egg, development, growth performance, survival rate, *Solea aegyptiaca*

INTRODUCTION

The common sole *Solea solea* and the Egyptian sole *S. aegyptiaca* (Family: Soleidae) are the most important sole species that occurs in the Egyptian waters. The common sole is highly appreciated fish by the Egyptians especially in the coastal communities because of its high quality flesh and is one of the commercially important fish in Egypt providing up to 90 million LE annually (Mehanna, 2014). The Egyptian sole (*Solea aegyptiaca*) is the most common species of soles that contributed about 6.5% of the total catch of trawl fishery, forming about 13% of the gross revenue of the trawling (Mehanna, 2007). Kariman (2009) recorded that catch composition of sole species during summer and winter seasons in Lake Qarun were more than 50 and 35%, respectively.

The rearing methods for this species have been well documented (Dinis *et al.,* 1999), although high mortalities during the weaning phase possibly due to inadequate nutrition and pathological problems (Zarza *et al.,* 2003) as well as poor success obtaining eggs from captive breeders (Anguis and Canavate, 2005) are constraints for the continued development of the industry. Senegalese sole larvae are fed on live prey (rotifer, *Brachionus plicatilis* and *Artemia* sp.) during the first 40 days after hatching; although earlier weaning on inert feeds has been attempted (Canavate and Fernandez-Diaz, 1999 and Dinis *et al.*, 1999). The nutritional value of live prey is, therefore, a key factor in the success of larval rearing. Indeed, exhibits good growth during the larval period when fed on live prey (Vazquez *et al.,* 1994 and Dinis and Reis, 1995); the weaning, switch from live prey to compound diet feeding, induces poor growth and mortality (Dinis, 1992 and Marin-Magan *et al.,* 1995), weaning and metamorphosis which are accompanied by increased mortality rates (Rueda-Jasso *et al.,* 2005). Standard feeding regimens during these periods represent a bottleneck for fish farmers due to the required administration to young larvae of live-feed usually characterized by: 1) variable availability and price fluctuations of *Artemia* cysts (Callan *et al.*,

2003), which can reach 700% (Moretti *et al.,* 2005); and 2) poor hygienic conditions and high levels of pathogenic bacteria (Olafsen, 2001 and Olsen *et al.,* 2000). Metamorphosis is a crucial developmental phase in flatfish species. The transformation from a symmetric pelagic larvae to an asymmetric benthic juvenile most conspicuously involves eye migration and craniofacial remodeling. Other transformations are recalibration of vision, changes in skin pigmentation and scale patterns, body shape, digestive tract and feeding behavior (Klaren *et al.,* 2008).

Stocking density is an important parameter in fish culture, not only because it has strong implications on growth performance, but also because it can affect fish welfare (Ellis *et al.,* 2005 and Turnbull *et al.,* 2005) and has an economical impact. Social interactions could be behind the differences observed in growth efficiency of several fish species.

Photoperiod was pointed out by Imsland *et al.* (2003) as one of the major researches needed in order to improve the rearing conditions of *S. senegalensis*. Most cultured marine fish species in Europe have been investigated for their larval response to photoperiod, as reported for *Dicentrarchus labrax* (Barahona-Fernandes, 1979), *Solea solea* (Fuchs, 1978 and Ramos, 1986), *Sparus aurata* (Chatain and Ounais-Guschemann, 1991) and *Gadus morhua* (Puvanendran and Brown, 2002). Many studies have shown that light manipulation (photoperiodism) can be used to modulate fish growth and sexual maturation in fish culture. Photoperiodism has also been demonstrated to compromise fish welfare in aquaculture (Hastein, 2004; Burgos *et al.,* 2004; Huntingford *et al.,* 2006 and Stevenson, 2007), which often leads to mortality. Different species of fish at different stages of life respond to different photoperiods for their growth, gonadal maturation, spawning, and feeding rhythms. Different photoperiods will also impair the welfare of fish and cause mortality in different species of fish at different stages of their life (Mustapha *et al.,* 2014).

The aim of the present study was to assess the overall affects of stocking density, photoperiod and feeding on poultry egg on the growth and

development of the Egyptian sole (*Solea aegyptiaca*) during early life stages.

MATERIAL AND METHODS

Larvae- rearing conditions.

The Egyptian sole, *Solea aegyptiaca* larvae used in the present study were obtained from the experiment of spawning conducted in National Institute of Oceanography and Fisheries (NIOF), Shakshouk Fish Research Station, El-Fayoum Governorate during the period from 14/12/2014 to 14/4/2015. The water used in these trials were obtained from Lake Qaroun and filtered through plankton net 50 µ mesh size. Larvae were collected from the date of the emergence of larvae in spawning tanks, and incubated in tanks from 1 to 3 days after hatching (DAH) under temperature 18 °C and then transported to the experimental rearing tanks. Larvae were collected by plankton net 150 µ mesh size. Larvae rearing was following up from 4 DAH to metamorphosis stage. For each tanks, continuous aeration was gently. The average water quality criteria of all experimental rearing larvae are presented in Table (1). About 30% (for stocking density and photoperiod trials) and 65% (for feeding on poultry egg trial**)** of water aquarium was changed twice every day. This experiment was carried out in 2 replicates for each treatment.

Table 1. Average values of water quality parameters during experiments period (Mean±S.E).

Parameters	Experiments larval rearing		
	Stocking density	**Photoperiod**	**Feeding on poultry egg**
Temperature, °C	18.15±0.65	17.40±0.90	17.65±1.15
pH	8.29±0.05	8.33±0.10	7.94±0.42
Salinity, ‰	31.5±1.5	31.5±1.5	31.5±1.5
Dissolved oxygen, mg/l	7.4±0.4	7.40±0.4	8.0±0.5
Total ammonia, mg/l	0.74±0.03	0.74±0.03	1.66±0.91
Un-ionized ammonia, mg/l	0.039±0.002	0.051±0.016	0.081±0.042
Nitrite, mg/l	0.321±0.007	0.320±0.006	0.434±0.113
Nitrate, mg/l	1.24±0.06	1.22±0.03	1.27±0.08

Culture of live food.

The live food organisms used in this study were the microalgae *Nannochloropsis oculata*, the rotifer (*Brachionus plicatilus*) and *Artemia*.

The golden unicellular alga *Nannochloropsis oculata*, obtained from the National Institute of Oceanography and Fisheries, Marine Hatchery Lab (Alexandria- Egypt). The culture of *Nannochloropsis oculata* was grown in glass flasks (capacity 1 liter), at increased growth transported to transparent plastic bags from 20 to 40 L water capacity. The water used in microalgae culture was filtered through plankton filter 1 µ mesh size. The cultures were grown under controlled laboratory conditions at temperature (20°C±3°C), salinity was 33‰, pH was from 8.11 to 8.27, continuous aeration was vigorous, illumination for alga culture was provided by fluorescent lights (24 h light). Each flask was regularly swirled daily by hand to detach adhered algal cells from the walls of flasks. *Nannochloropsis oculata* was fertilized with media as 1 ml Super phosphate solution (5g Super phosphate in 1 L distilled water) and 1 ml Urea solution (5g Urea in 1 L distilled water) per 1 L of *Nannochloropsis oculata* per day.

The rotifer, *Brachionus plicatilus*, was obtained from the National Institute of Oceanography and Fisheries, Marine Hatchery Lab (Alexandria-Egypt). Rotifer cultures were carried out using filtered saline water by plankton filter 30 µ mesh size at 33‰ salinity using *Nannochloropsis oculata* as exclusive food (5×10^5 cell/ml). The culture of rotifers was grown in tanks (capacity 500 liter). Rotifer cultures were carried out under defined illumination with a photoperiod of 12:12 light:dark, pH ranged from 8.11 to 8.27, temperature ranged from 20 to 23°C, continuous aeration should be gently. The maintenance of rotifers was depending on the enrichment with microalgae at a very high density to obtain high density of rotifers. Once the water containing the rotifer cultures become clear, the rotifers will be in their highest density and again must be fed. The cultures received a continuously supply of commercial yeast.

Artemia cysts (*Artemia* International LLC, U.S.A) were brought from commercial market in Cairo, Egypt and they "hatched " with the addition of saline water as the following: A rectangular hatching glass aquaria (25 L water capacity) equipped with continuous aeration and heater with thermostat (JAGER 3609 Aquarium Heater, Automatic heater, Germany). Water salinity was 33‰. The heater with thermostat was added to keep water temperature at 25°C. The aeration was set on high rate. Water pH was from 8.11 to 8.27. The weight of cysts determined before adding to the glass aquaria at a density of 4 g per 14 liter. *Artemia* hatching were carried out under defined illumination with a photoperiod of 24 light. Under these conditions, the cysts hatched after about 20 hours. The hatched *Artemia* was buoyant on the surface water.

Larvae feeding regime.

Larvae opened their mouth at about 3±1 DAH and started to feed. Larvae was transported to the larval rearing tanks, after the start of the exogenous feeding. *Solea aegyptiaca* larvae were rearing from 4 DAH until metamorphosis stage, adopting a feeding regime based on live food only. Larvae were fed on Rotifer (20 individuals (ind.)/ml) from 3 DAH until 15 DAH. From 8 DAH, larvae fed on newly hatched *Artemia* nauplii (10 ind./ml). *Artemia* nauplii were introduced at 8 DAH and their density was gradually increased, becoming the only prey offered from 16 DAH. Feed was offered by hand at three meals/day (9:00, 13:00 and 16:00 h). Microalgae (*Nannochloropsis oculata*) at a final concentration of $5x10^5$ cells/ml were also added to the rearing tanks from first feeding. By the age of metamorphosis stage, it fed on *Artemia* metanauplii (8-12 ind./ml).

The first experiment: Effect of stocking density.

The first experiment was conducted to investigate the effect of stocking density, (50, 80, 110 and 140 larvae/ liter) on growth performance, metamorphosis stage and survival rate of *Solea aegyptiaca* larvae. Larvae of 2.73 ±0.01 mm initial mean length, were randomly distributed into the experimental circular plastic tanks of 25 L water capacity by using 8

experimental plastic tanks. Feeding regime based on live food shown in Table (2).

The second experiment: Effect of photoperiod.

The second experiment was conducted to investigate the effect of photoperiods (light, L: dark, D) (6L:18D, 12L:12D, 18L:6D and 24L:0D) on growth performance, metamorphosis stage and survival rate of *Solea aegyptiaca* larvae. Larvae of 2.74 ±0.01 mm initial mean length, were randomly distributed into the experimental rectangular glass aquaria of 25 L water capacity by using 8 experimental glass aquaria at a density of 80 larvae per liter. The photoperiod was controlled by a timer (Theben AG D-72401 Haigerloch, Germany). Feeding regime based on live food shown in Table (3).

The third experiment: Effect of feeding on poultry egg.

The third experiment was conducted to investigate the effect of feeding on poultry egg, (whole egg, egg white, egg yolk and boiled egg yolk) compare to live food organisms (Rotifer and *Artemia*) on growth performance, metamorphosis stage and survival rate of *Solea aegyptiaca* larvae. Larvae of 2.72 ±0.01 mm initial mean length, were randomly distributed into the experimental circular plastic tanks of 25 L water capacity by using 10 experimental plastic tanks at a density of 80 larvae per liter. Feeding regime based on live food shown in Table (3) and feeding on poultry egg shown in Table (4).

Table 2. Feeding regime based on live food organisms under effect of stocking density of *Solea aegyptiaca* larvae during experimental period.

Items	Number/ ml	Amount / tank	Number/ tank
Rotifer, 3- 7 DAH			
Density 50 larvae /L	20 ind./ml	0.625 liter/ tank	12500 ind./ tank
Density 80 larvae /L	20 ind./ml	1 liter/ tank	20000 ind./ tank
Density 110 larvae /L	20 ind./ml	1.375 liter/ tank	27500 ind./ tank
Density 140 larvae /L	20 ind./ml	1.75 liter/ tank	35000 ind./ tank
Rotifer, 8- 15 DAH			
Density 50 larvae /L	20 ind./ml	0.313 liter/ tank	6260 ind./ tank
Density 80 larvae /L	20 ind./ml	0.5 liter/ tank	10000 ind./ tank
Density 110 larvae /L	20 ind./ml	0.688 liter/ tank	13760 ind./ tank
Density 140 larvae /L	20 ind./ml	0.875 liter/ tank	17500 ind./ tank
***Artemia* 8-15 DAH**			
Density 50 larvae /L	10 ind./ml	0.625 liter/ tank	6250 ind./ tank
Density 80 larvae /L	10 ind./ml	1 liter/ tank	10000 ind./ tank
Density 110 larvae /L	10 ind./ml	1.375 liter/ tank	13750 ind./ tank
Density 140 larvae /L	10 ind./ml	1.75 liter/ tank	17500 ind./ tank
***Artemia* 16 DAH- metamorphosis stage**			
Density 50 larvae /L	10 ind./ml	1.25 liter/ tank	12500 ind./ tank
Density 80 larvae /L	10 ind./ml	2 liter/ tank	20000 ind./ tank
Density 110 larvae /L	10 ind./ml	2.75 liter/ tank	27500 ind./ tank
Density 140 larvae /L	10 ind./ml	3.5 liter/ tank	35000 ind./ tank

Table 3. Feeding regime based on live food organisms.

days	Number/ ml	Amount/ tank	Number/ tank
Rotifer, *Brachionus plicatilus*			
3- 7 DAH	20 ind./ml	1 liter/ tank	20000 ind./ tank
8-15 DAH	20 ind./ml	0.5 liter/ tank	10000 ind./ tank
***Artemia* nauplii**			
8-15 DAH	10 ind./ml	1 liter/ tank	10000 ind./ tank
16 DAH - metamorphosis stage	10 ind./ml	2 liter/ tank	20000 ind./ tank

Table 4. Feeding regime based on poultry egg.

poultry egg	diameter	Number/ ml	Amount / tank	Number/ tank
whole egg	0.5-1µm	400 grains/ ml	50 ml/ tank	20000 grains /tank
egg white	0.5 µm	375 grains / ml	53.5 ml/ tank	20062 grains /tank
egg yolk	0.5 µm	375 grains / ml	53.5 ml/ tank	20062 grains /tank
boiled egg yolk	100-600 µm	5000 grains/ ml	4 ml/ tank	20000 grains /tank

Parameters measurements.

At the end of the experiment, growth performance and survival rate were calculated as follows:

- Total length gain (mm) = final length, mm - initial length, mm.
- Average daily length gain (mm/day) = average length gain, mm/ experimental period, day.
- Condition factor (mg/mm^3) = (wet weight)/ (total length^3) ×100.
- Survival rate (SR,%) = (number of fish at end/ number of fish at start) × 100.

Water quality analysis.

Water temperature and pH were measured daily by Combined meter (pH/ EC/ TDS/ temperature, Mi 805). Salinity was measured daily by Refractometer (VITAL Sine SR-6, China). Dissolved oxygen (DO) concentration was determined titrimetrically according to the modified Winkler, full-bottle technique (Method 360.2; EPA, 1983). Water ammonia, nitrite and nitrate were determined by using Spectrophotometer model (LKB Bichrom UV visible spectrophotometer) according to the method described by APHA (1992). To determine un-ionized ammonia concentration, multiply total ammonia concentration by the percentage which is closest to the observed temperature and pH of the water sample (Swann, 1997).

Estimation of eye migration stage .

Metamorphosis degree was evaluated on 20 larvae/ tank. Degrees of metamorphosis were divided into 5 phases: 1) symmetrical left and right eye position; 2) an asymmetrical position of the left eye and right eye, the left eye starts to migrate; 3) the migrating eye reaches at maximum the midline of the dorsal surface; 4) the migrating eye can be seen from the right ocular side or migrates within the dorsal side; 5) eye translocation is completed and the orbital arch is visible.

Statistical analysis.

The data were analyzed by one-way ANOVA and significant differences were determined by Duncan Waller Multiple Range Test at 5% level using SPSS Statistical Package Program (SPSS, 2008) 17, released version.

RESULTS

Generally, the total length of the larvae throughout the period of the study has been increased gradually with the larval development. Survival rate from hatching till 7 DAH was high in all trials larval rearing under all treatments, but survival rates decreased progressively after 9-15 DAH from started day until finished metamorphosis stage in all treatments in all trials larvae rearing. Overall, initial average length of the larvae using in all trials larvae rearing was ranged from 2.70 to 2.75 mm (at age 4 DAH) among treatments, with no significant difference ($P \leq 0.05$). At the end of the trials have been larvae successfully completed metamorphosis.

Developmental stages of the larvae and post larvae. At the 3 days in all trials, the larvae were wholly endogenous. It depends on the nutrients storage on the yolk sac. At age about 4 DAH transition of larval feeding from endogenous to exogenous nutrition. From about 8 DAH, larval nutrition was entirely exogenous. Then, start of metamorphosis from 15-20 days and continued until metamorphosis is completed when the left eye transferred to right side of the head at about 25-45 DAH.

Metamorphosis in the eye and caudal fin when started, the larvae become asymmetric. The eye migration is beginning when the left eye is shifting to the dorsal midline of the head. The most representative stages of metamorphosis are when the left eye reaches the dorsal midline of the head; the larvae begin to change their swimming from vertical to benthic. Transparency of the body begins to reduce skin pigmentation intensifies. The left eye began to migrate to the other side, in accordance with the normal changes which take place in this species. At the age of 25-45 DAH, the larvae transformed symmetric floating

larvae to asymmetric benthic juvenile. Both eyes now in the right side of the body and the juvenile assumes the benthic behavior typical of flatfishes. The pattern of pigmentation of sub-adult was becoming apparent. It becomes morphologically typical the adult one; a concentration of the pigment is distributed in all over the body in just one side of the flatfish. Metamorphosis was different according to the variability in photoperiod, stocking density and regime feeding in the following trials larvae rearing.

The first experiment: Effect of stocking density on growth performance, metamorphosis stage and survival rate.

Results of growth performance, metamorphosis stage and survival rate for larvae reared in different stocking density are present in Table (5). From table (5), it was noticed that stocking density of 110 larvae/liter led to relatively higher survival rate compared to the other stocking densities, with significant differences ($P \leq 0.05$) were observed. The results showed that insignificant differences ($P \leq 0.05$) were obtained in all growth performance parameters between treatments. At the end of the trial (at completed the metamorphosis), average final weight and final length of larvae were 13.00, 10.75, 13.35, 10.60 mg and 11.50, 10.50, 11.00, 9.90 mm, for larvae reared in stocking densities 50, 80, 110 and 140 larvae/liter, respectively. Metamorphosis of the sampled speciemens were completed at 36.0, 36.5, 35.5, 36.0 DAH for the same treatments, respectively. The results indicated that the growth rate of larvae was similarly in all stocking densities, without any statistical difference between treatments (50, 80, 110 and 140 larvae/liter) under experimental conditions.

Table 5. Effect of stocking density on growth performance, metamorphosis stage and survival rate of *Solea aegyptiaca* larvae.

Items	Stocking density (larvae/ liter)				SED*
	50	80	110	140	
Initial aveg. Length, mm/larvae	2.74	2.74	2.72	2.72	0.027
Final aveg. Length, mm/larvae	11.50	10.50	11.00	9.90	0.869
Total length gain, mm/larvae	8.76	7.77	8.29	7.18	0.887
Average daily length gain, mm/larvae/day	0.25	0.21	0.23	0.20	0.032
Final aveg. Weight, mg/larvae	13.00	10.75	13.35	10.60	2.332
Condition factor, mg/mm^3	0.86	0.93	1.00	1.09	0.148
Metamorphosis stage, day	36.0	36.5	35.5	36.0	1.500
Survival rate, %	7.55^{b}	8.53^{b}	10.60^{a}	7.38^{b}	0.551

$^{a-b}$Average in the same row having different superscripts are differ significantly (P≤0.05). * SED is the standard error of difference

The second experiment: Effect of photoperiod on growth performance, metamorphosis stage and survival rate.

Results of growth performance, metamorphosis stage and survival rate for larvae reared in different photoperiods are present in Table (6). Larvae exposed to different lights showed significant differences (P≤0.05) in survival rate between treatments, larvae exposed to photoperiods (12L:12D, 18L:6D and 24L:0D) led to a higher survival rate compared to 6L:18D. The results showed that significant differences (P≤0.05) were obtained in all growth performance parameters between treatments except the condition factor. Larvae exposed to photoperiods (12L:12D, 18L:6D and 24L:0D) led to higher length compared to 6L:18D. Final weight values were highest with 24L:0D followed 18L:6D and 12L:12D then 6L:18D. Larvae reared under photoperiods 6L:18D, 12L:12D, 18L:6D and 24L:0D completed the metamorphosis at 45, 29, 25.5 and 27 DAH, respectively. The metamorphosis of group which reared under 18L:6D was the most fast in the other photoperiods, with insignificant differences between (12L:12D, 18L:6D and 24L:0D). The results indicated that photoperiods of 18L:6D and 24L:0D improved the growth rate of larvae without statistical difference between (12L:12D, 18L:6D and 24L:0D) under experimental conditions.

Table 6. Effect of photoperiod on growth performance, metamorphosis stage and survival rate of *Solea aegyptiaca* larvae.

Items	Photoperiod (light (L): Dark (D))				SED*
	6L:18D	12L:12D	18L:6D	24L:0D	
Initial aveg. Length, mm/larvae	2.73	2.73	2.74	2.75	0.017
Final aveg. Length, mm/larvae	6.15^{b}	8.70^{a}	9.50^{a}	9.45^{a}	0.550
Total length gain, mm/larvae	3.43^{b}	5.98^{a}	6.76^{a}	6.70^{a}	0.541
Average daily length gain, mm/larvae/day	0.08^{b}	0.21^{a}	0.27^{a}	0.25^{a}	0.032
Final aveg. Weight, mg/larvae	2.45^{b}	7.10^{ab}	9.90^{ab}	10.85^{a}	2.747
Condition factor, mg/mm^3	1.06	1.06	1.14	1.28	0.247
Metamorphosis stage, day	45.0^{a}	29.0^{b}	25.5^{b}	27.0^{b}	1.414
Survival rate, %	1.50^{b}	7.10^{a}	7.35^{a}	7.38^{a}	0.192

$^{a-b}$Average in the same row having different superscripts are differ significantly ($P \leq 0.05$). * SED is the standard error of difference

The third experiment: Effect of feeding on poultry egg on growth performance, metamorphosis stage and survival rate.

Results of growth performance parameters, metamorphosis stage and survival rate of larvae fed with the different feed are shown in Table (7). Survival rate values were relatively highest with fed on egg white and fed on live feed (Rotifer and *Artemia*), with significant differences were observed. The results showed that significant differences ($P \leq 0.05$) were obtained in all growth performance parameters between treatments except the condition factor. At the end of the trial (at completed the metamorphosis), average final weight and final length of larvae were 15.90, 2.75, 5.35, 2.65, 2.85 mg and 10.00, 6.60, 7.40, 6.50, 6.50 mm, in groups of larvae fed on live feed, whole egg, egg white, egg yolk and boiled egg yolk, respectively. Metamorphosis of the sampled speciemens were completed at 36.0, 42.5, 41.5, 43.00, 42.5 DAH for the same treatments, respectively, the slower development of larvae grown at feeding on poultry egg. Larvae fed on live feed showed statistically higher growth rate comparing to all the other groups. The highest growth rate was observed in larvae fed on live feed, while there was no significant difference among the other experimental groups of larvae. These results indicated that the best growth rate of larvae was obtained

at feeding on live feed (Rotifer and *Artemia*) then feeding on egg white under experimental conditions.

Table 10. Effect of feeding on poultry egg on growth performance, metamorphosis stage and survival rate of *Solea aegyptiaca* larvae.

Items	T_1	T_2	T_3	T_4	T_5	SED*
Initial aveg. Length, mm/larvae	2.73	2.72	2.72	2.71	2.72	0.033
Final aveg. Length, mm/larvae	10.00^{a}	6.60^{b}	7.40^{b}	6.50^{b}	6.50^{b}	0.639
Total length gain, mm/larvae	7.27^{a}	3.89^{b}	4.69^{b}	3.80^{b}	3.78^{b}	0.651
Average daily length gain, mm/larvae/day	0.20^{a}	0.09^{b}	0.11^{b}	0.09^{b}	0.09^{b}	0.017
Final aveg. Weight, mg/larvae	15.90^{a}	2.75^{c}	5.35^{b}	2.65^{c}	2.85^{c}	0.940
Condition factor, mg/mm^3	1.59	0.99	1.35	0.99	1.04	0.247
Metamorphosis stage, day	36.0^{b}	42.5^{a}	41.5^{a}	43.0^{a}	42.5^{a}	0.837
Survival rate, %	10.70^{a}	9.40^{b}	11.38^{a}	7.30^{c}	7.08^{c}	0.292

$^{a-c}$Average in the same row having different superscripts are differ significantly ($P \leq 0.05$). * SED is the standard error of difference

- T_1 - Live feed, T_2 - Whole egg, T_3 - Egg white, T_4 - Egg yolk, T_5 - Boiled egg yolk

DISCUSSION

Generally, in the present trials the larval survival rates (7-11.38%) were lower than range survival rates in the previous studies (74.5-81%) for *Solea senegalensis* (Canavate *et al.*, 2006), 44-65% for *Solea senegalensis* (Salas-Leiton *et al.*, 2012), 43-56% for *Solea solea* (Bonaldo *et al.*, 2011), 40% for *Solea solea* (Palazzi *et al.*, 2006). This may be attributed to pollution by coliform bacteria in the water source of Lake Qaroun (total coliforms 190, fecal coliforms 140, fecal streptococci 260/100 ml). Also, it may be related to differences in larval rearing conditions.

Nutritional unbalance (Gisbert *et al.*, 2008) and environmental conditions such as pollution (Sun *et al.*, 2009), extreme temperature (Okamura *et al.*, 2007) and physical stress (Morrison and Mac-Donald 1995) have been reported as likely causing jaw deformities. Abnormalities in jaw development, and the resulting inability to feed (Morrison and Mac-Donald 1995) which may affect the overall larval survival. From previous studies the survival rate of larvae depends on environmental conditions such

as photoperiods, light intensity, salinity, temperature, stocking density, feed quality, feeding regime, rearing system (aeration and water exchange), tank size and tank colour.

In the present trials, un-ionized ammonia concentration ranged from 0.039 to 0.081 mg/l. For salmonid fishes, it is recommended that the concentration of un-ionized ammonia not exceed 0.0125 to 0.02 mg/l to maintain health of the fish, however, the toxic concentrations of un-ionized ammonia (NH3) for trout are about 0.32 mg/l for rainbow trout, but 1.50-3.10 for channel catfish (Boyd, 1990). Thus, assuming un-ionized ammonia within the tolerance levels for *Solea aegyptiaca*, but increasing un-ionized ammonia may cause deterioration in water quality leading to stressful conditions.

The metamorphosis in flatfish is related to the change from the pelagic to benthic habitat, and it implies important changes in fish physiology (Fernandez-Diaz *et al.*, 2001). The transformation occurs at a wide range of sizes depending on species and environmental circumstances (Policansky, 1982; Ottesen and Bolla, 1998). Whether age or size are key factors in starting metamorphosis is a question that has been considered in several studies with other species, such as Atlantic halibut larvae (Ottesen and Bolla, 1998). In addition, this question is important in laboratory populations in which growth and therefore, the size of larvae and successful metamorphosis depend on the rearing conditions (Fernandez-Diaz *et al.*, 2001). Parameters such as stocking density, salinity, temperature, feeding or light have been observed to affect the development and metamorphosis process in flatfish (Bolla and Holmefjord, 1988 and Daniels *et al.*, 1996).

Effect of stocking density.

Stocking densities is one important factors affecting growth in fish Salas-Leiton *et al.* (2010). Studies on larval rearing of sole fish were used stocking density as followed: stocking density 7 larvae/ liter (2000 larvae/ 280 liter) for *Solea solea* (Bonaldo *et al.*, 2011), stocking density 40 larvae/ liter for *Solea senegalensis* (Canavate *et al.*, 2006 and Salas-Leiton *et al.*,

2012), stocking density ranged from 50-70 larvae/ liter for *Solea vulgaris* (Assem *et al.,* 2012 and El-Dahhar *et al.,* 2013), stocking density ranged from 150-200 larvae/ liter for *Solea solea* (Palazzi *et al.*, 2006).

In the present study, four different stocking densities (50, 80, 110, 140 larvae/ liter) were tested for *Solea aegyptiaca* larvae. The growth rate of larvae was similarly in all stocking densities, without any statistical difference between treatments under experimental conditions. These results similar with the findings of Salas-Leiton *et al.* (2008) who studied the effect of four stocking densities between 2 and 30 kg/m^2 of Senegalese sole that did not find any significant differences in biomass production or growth rates. Also, Salas-Leiton *et al.* (2010) studied the effect of stocking density (7 and 30 Kg/m^2) on the growth of Senegalese sole (*Solea senegalensis*) juveniles for 60 days, and there were no differences in SGR between densities. In addition, Hatziathanasiou *et al.* (2002) reported that, the effect of stocking densities (50, 100, 150 and 200 larvae/ liter) on the growth of sea bass larvae for 30 days, results indicate that stocking density did not affect growth of larvae with little higher with 100 larvae/ liter than the other densities. Moreover, Salama (2007) studied the effect of stocking densities (20, 40, 60, and 80 larvae/liter) on the growth of Asian sea bass (*Lates calcarifer*) larvae for 20 days, showed that no significant size differences detected at the end of the larval culture period. Also, Saoud *et al.* (2007) studied the effect of stocking densities (10, 20, 30, and 40 fish per 52-l aquarium) on the growth of rabbitfish (*Siganus rivulatus*) juveniles for 56 days, the results suggested that there was no apparent effect of stocking density at the levels tested on the growth of juvenile rabbitfish. As well as, Moradyan *et al.* (2012) studied the effect of stocking densities (40, 60, and 80 fry/L) on the growth of Rainbow trout (*Oncorhynchus mykiss*) fry for 45 days, the stocking density did not significantly affected final weight, specific growth rates and condition factors at the end of the study. Also, Gomes *et al.* (2006) working with tambaqui (*Colossoma macropomum*).

These researchers stocked fish at 20, 30, 40 and 50 fish/m^3 and they found no differences in growth among treatments.

On the other hand, the results disagree with results obtained by Schram *et al*. (2006) studied the effect of stocking density (0.5, 1.1, 5.1, 7.4, 10.2 and 12 kg/m^2) on the growth of Dover sole for 55 days, and they found that the SGR was decreasing with increasing stocking density. Also, Hatziathanasiou *et al.* (2002) reported that, effect of stocking densities on growth of sea bass post-larvae (5, 10, 15 and 20 post-larvae/ liter), growth performance fluctuated between the lowest value recorded in the group of 10 fish/L and the highest value in that of 5 fish/L. El-Sayed (2002) also studied the effect of stocking density (3, 5, 10, 15 and 20 fry/L) on the growth of Nile tilapia and they found that best performance was achieved at fish stocked at 3 fry/L . El-Saidy and Gaber (2002) found that mean final weight and SGR of Tilapia (*O. niloticus*) significantly higher at the lower stocking density. Gibtan *et al.* (2008) who studied the effects of stocking density (50, 100, 150 and 200/m^3) on Nile tilapia *O. niloticus* under cage culture and they found that the highest weight was attained at a density of 50 fish/m^3. Also, Mensah *et al.*, (2013) who studied the effect of stocking density (1000, 1500 and 2000 fry/m^2) on the growth performances of Nile tilapia and they found that final body weight gain was significantly higher at a density of 1000 fry/m^2. Moreover, Kapinga *et al.* (2014) studied the effect of stocking density (3 and 13 fish/m^2) on the growth of Nile tilapia for 150 days. There was significantly higher fish growth performance in 3 fish/m^2. In addition, Bagum *et al* (2015) studied the effect of stocking densities (1200, 1700 and 2200 fry/m^2) on the growth of Nile tilapia (*Oreochromis niloticus*) fry for 28 days, the growth performances was significantly higher in 1200 fry/m^2 than those obtained from 1700 and 2200 fry/m^2, respectively.

In this study, stocking density of 110 larvae/liter led to relatively higher survival rate compared to the other stocking densities, with significant differences between treatments. These results similar with the findings of

Hatziathanasiou *et al.* (2002) reported that, the effect of stocking densities on survival of sea bass larvae (50, 100, 150 and 200 larvae/ liter). Results indicate that stocking density did not affect survival rate of larvae with little higher with 200 larvae/ liter than the other densities. On the other hand, Schram *et al*. (2006) showed that, survival rate decreased significantly with increasing stocking density for Dover sole. Also, Hatziathanasiou *et al.* (2002) showed that, survival rate was significantly higher in low stocking densities for sea bass post-larvae. In addition, Gomes *et al.* (2006) found no differences of stocking density on the survival rate of tambaqui (*Colossoma macropomum*). Also, Saoud *et al.* (2007) showed that, no apparent effect of stocking density on the survival rate of rabbitfish juvenile. Also, Salama (2007) showed that survival rate was significantly higher at a stocking density of 20 larvae/L, however there were no significant differences among survival rates 40, 60 and 80 larvae/L for Asian sea bass (*Lates calcarifer*). Moreover, Moradyan *et al.* (2012) showed that, survival rate was significantly lower at high stocking density for Rainbow trout (*Oncorhynchus mykiss*).

The results of our study showed that growth of *Solea aegyptiaca* larvae was reasonably good at different stocking densities (from 50 to 140 larvae/ liter). The findings may indicate that stocking density might have a limited effect on larvae growth during the intensive larval rearing period. The higher survival rate at high stocking density could be attributed to diminished social dominance (Kapinga *et al.,* 2014). The results of our study show that *Solea aegyptiaca* larvae (4-36 DAH) can be maintained at densities as high as 110 larvae/ liter with no negative effect on survival and growth rates with higher survival rate compared to the other stocking densities. In brook charr, Hardy and Audet (1990) observed that growth performances were not affected by density and interpreted this as an example of good application of rearing management and high water quality.

Effect of photoperiod.

Different conditions of photoperiod affected the development and growth performance of *Solea aegyptiaca* larvae. In the present study, four different photoperiods (6L:18D, 12L:12D, 18L:6D and 24L:0D) were tested for *Solea aegyptiaca* larvae. Larvae exposed to photoperiods (12L:12D, 18L:6D and 24L:0D) led to higher length compared to 6L:18D. The highest final weight was observed in 24L:0D followed 18L:6D and 12L:12D then 6L:18D. The results indicated that the best growth performance of larvae at photoperiods of 18L: 6D and 24L: 0D.

These results similar with the findings of Canavate *et al.* (2006) reported that, growth of Senegal sole larvae was not significantly affected by photoperiods 14L:10D, 10L:14D and 24L:0D. Similar growth were reported for larvae of the close relative species *S. solea* reared in 18L:6D or 24L:0D (Fuchs, 1978), and 15L:9D or 24L:0D (Ramos, 1986). However, both authors pointed out a slightly lower total length for larvae reared in 12L:12D and 11L:13D, respectively. Also, Leal *et al.* (2000) indicated similar lengths at 18 DAH for *S. senegalensis* larvae reared in 12 h or 24 h light. Reports for flatfish also indicate some growth improvements under longer photoperiods, although differences were never very high. Growth of 15 d-old *Paralichthys lethostigma* larvae was higher in 24L:0D and 18L:6D, in comparison to 12L:12D and 6L:18D (Moustakas *et al.*, 2004). Growth of the summer flounder (*Paralichthys dentatus*) larvae was not affected when photoperiod was set to 24L:0D or 16L:8D (Huber *et al.*, 1999). Reduction of photoperiod from 24L:0D to 12L:12D decreased growth in 20 d-old *R. tapirina*, but 18L:6D produced similar results than permanent light (Hart *et al.*, 1996). Growth was also lower for *Hippoglossus hippoglossus* larvae reared in 12L: 12D, in relation to 24L:0D (Solbakken and Pitmann, 2004). However, these authors found eye migration to initiate earlier under the 12L: 12D light regime. On the other hand, Tuckey and Smith (2001) found no photoperiod effect on growth after 54 days of rearing post-hatched *P. lethostigma*.

Several species have been shown to grow better under longer photoperiods, as it is the case of *S. aurata* (Tandler and Helps, 1985), *S. guttatus* (Duray and Kohno, 1988), *L. calcarifer* (Barlow *et al.*, 1995), *P. auratus* (Fielder *et al.*, 2002), *G. morhua* (Puvanendran and Brown, 2002) and *L. lineata* (Trotter *et al.*, 2003). There are, however, species which growth was not increased with longer photoperiods, as described for *Melanogrammus aeglefinus* (Downing and Litvak, 1999), and *Morone saxatilis* (Martin-Robichaud and Peterson, 1998). Barahona-Fernandes (1979) which showed that continuous light in sea bass larvae did not induce the best growth. The larvae of *Dentex dentex* also grew better in a 18L: 6D photoperiod than under permanent illumination (Abellan *et al.*, 2000).

The results of the current study, showed that the metamorphosis period completed between 25.5 and 29 DAH under photoperiods (12L:12D, 18L:6D and 24L:0D), it was faster than 6L:18D (45 DAH). But at 18L:6D results recorded the fastest metamorphosis (25.5 DAH). Similar results were obtained by Blanco-Vives *et al*. (2010) showed that, metamorphosis was not complete until 27 DAH under photoperiod 12L:12D for *Solea senegalensis*. Contrarily, Canavate *et al.* (2006) for *Solea senegalensis* larvae exhibited similar metamorphosis under photoperiods (14L:10D, 10L:14D and 24L:0D) and completed from 19 to 20 DAH.

In this study, *Solea aegyptiaca* larvae exhibited similar survival rate under photoperiods (12L:12D, 18L:6D and 24L:0D) and higher than 6L:18D. This agreed with reports from Canavate *et al.* (2006) for Senegal sole larvae exhibited similar survival rate under photoperiods 14L:10D, 10L:14D and 24L:0D, and Leal *et al.* (2000) for Senegal sole, and Fuchs (1978) for *S. solea*, and for the larvae of other cultured flatfish such as *R. tapirina* (Hart *et al.*, 1996), *P. dentatus* (Huber *et al.*, 1999) and *P. lethostigma* (Moustakas *et al.*, 2004). Survival rate was higher in *H. hippoglossus* reared in 24L:0D against 12L:12D (Solbakken and Pitmann, 2004). On the other hand, Blanco-Vives *et al.* (2010) showed that, *Solea senegalensis* larvae reared under continuous darkness and continuous light died 15 and 17 DAH respectively at the beginning of metamorphosis. In

addition, Tuckey and Smith (2001) found improved survival rate for *P. lethostigma* when photoperiod was decreased from 24L:0D to 10L:14D. Care must be taken when comparing photoperiod effects among species, since results are unavoidable influenced by varying experimental conditions among facilities. Interactions due to light intensity (Downing and Litvak, 1999; Henne *et al.*, 2001; Puvanendran and Brown, 2002 and Moustakas *et al.*, 2004), feed quality (Naess and Lie, 1998) or tank colour (Downing and Litvak, 1999).

Different rates of survival have been recorded in fish species cultured under different photoperiod regimes. For example, Giri *et al.* (2002) reported the lowest survival rate in a continuous 24-h dark/light regime (0L:24D) for *Wallago attu*. Burke *et al.* (2005) recorded higher survival rate in Arctic char cultured under a 24-h continuous photoperiod (24L:0D), while Aride *et al.* (2006) observed no mortality in tanbaqui (*Colossoma macroponum*) when cultured under 3 photoperiods of 24 h of light, 24 h of darkness and 10 h of light and 14 h of darkness. Shan *et al.* (2008) and Freitals *et al.* (2009) showed that photoperiodism affected the mortality of Miiuy croaker larvae *Miichthys miiuy* and pejerry larva *Odontesthes argentinensis*, respectively. The lowest survival rate was recorded in African catfish *Clarias gariepinus* when cultured in 24 h of continuous light (Mino *et al.*, 2008). Appelbaum and Kamler (2000) also reported that continuous light decreased survival rate in *Clarias gariepinus* larvae. Mustapha *et al.* (2014) recorded lowest survival rate in *O. niloticus* cultured under 0L:24 D and lowest survival rate in *C. gariepinus* under photoperiod (24L:0D).

In the present study, the better growth rate and faster metamorphosis of larvae were observed at photoperiods of 18L:6D and 24L:0D. Early pelagic stages of *Solea aegyptiaca* depended on light to capture rotifers. Rotifers and *Artemia* nauplii greatly differ in size, swimming activity and transparency, and these features may influence their detection capacity by larvae, particularly in a low or no light environment, as it has been described for *S. senegalensis* (Canavate *et al.*, 2006). In work (Mussi *et al.*, 2005) different feeding reactions were described for a planktivorous fish larvae depending on transparency of the

prey. There seems to be a synergistic effect between food availability and light that improves the growth rate of larvae (Boeuf and Le Bail, 1999). Permanent illumination improved growth rate of sole larvae. Increasing the duration of the visual feeding period is generally associated to a higher growth rate in cultured marine larval fish (Howell *et al.,* 1998). Photoperiod may also stimulate hormones controlled by pineal organs which are responsible for circadian rhythms (Ekstrom and Meissi, 1997).

In this study, the lowest survival rate, growth rate and slower development of larvae were observed at photoperiod of 6L:18D, as a possible consequence more reduced capacity of sole larvae to catch rotifers in the dark, larvae start to cease feeding at times coinciding with the onset of darkness, as it has been described for *S. senegalensis* (Canavate *et al.,* 2006). The unfavorable photoperiods manifested in stress conditions that caused injuries and affected swimming activity, behavior, coloration, and growth (Mustapha *et al.,* 2014). The unfavorable photoperiods could have also affected the immune systems of *O. niloticus* and *C. gariepinus* (Mustapha *et al.,* 2014), Burgos *et al.* (2004) reported that artificial photoperiods affect the immune system of Rainbow trout, leading to mortality.

Effect of feeding on poultry egg..

In the present study, five different feeds type (live food (Rotifer and *Artemia*) and poultry egg (whole egg, egg white, egg yolk and boiled egg yolk)) were tested for *Solea aegyptiaca* larvae. Larvae that fed on live food showed statistically higher growth rate compared to all the other groups. It had showed the slow growth of larvae that were fed on poultry egg. Survival rate values were relatively high with fed on egg white and fed on live food, with significant differences were observed.

Some time supplemented the initial diet of rotifers with fertilized sea urchin eggs or with a mixture of boiled egg yolk and finely-ground artificial feed (Nelson and Wilkins, 1994). Egg yolk, as a simple and available food, is

a major source of vitamins and minerals being used to feed fish larvae newly absorbed their yolk sac (Maleknejad *et al.*, 2014).

From this study, we showed that, larvae fed on live food recorded statistically higher growth rate compared to fed on poultry eggs. It might be related to the following: live feed are able to swim in the water column and thus they are constantly available to the larvae. While, poultry eggs tend to aggregate on the water surface or, more commonly, sink quickly to the bottom, and thus they are normally less available to the larvae than the live feed. In addition, the movement of live feed in the water is likely to stimulate larval feeding responses. Since evolutionary history has probably adapted them to attack moving prey in nature. Also, poultry eggs are generally capable of moving only in a downward direction, towards the bottom. Finally, live prey, with a thin exoskeleton and high water content, may be more palatable to the larvae once were taken into the mouth, compared to the hard, dry poultry eggs. Any foods must enter the mouth whole (i.e. the larva's mouth gape must be of sufficient size for particle ingestion to occur) and they are quickly either accepted or rejected on the basis of palatability.

In conclusion, from the results of the present study, showed that growth rate of *Solea aegyptiaca* larvae was reasonably good at different stocking densities (from 50 to 140 larvae/ liter), stocking density of 110 larvae/liter led to relatively higher survival rate compared to the other stocking densities. The best growth performance of larvae was achieved at photoperiods of 18L:6D and 24L:0D. Larvae that fed on live food showed statistically higher growth rate compared to fed on poultry egg under experimental conditions. So, it is recommended to *Solea aegyptiaca* larval rearing under stocking density (110 larvae/ liter), reared of larvae under photoperiod of 18L:6D and feeding of larvae on live food (Rotifer and *Artemia*).

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تأثير كثافة التخزين ، الفترة الضوئية والتغذية على بيض الدجاج على نمو وتطور يرقات أسماك موسى المصرية

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الملخص العربي

أجريت هذه الدراسة لتقييم تأثير كل من كثافة التخزين، الفترة الضوئية والتغذية على بيض الدجاج على نمو وتطور يرقات أسماك موسى المصرية (*Solea aegyptiaca*) . التجربة الأولى أختبرت أربعة كثافات مختلفة (٥٠، ٨٠، ١١٠، ١٤٠ يرقة/لتر) وأوضحت النتائج أنه لا يوجد اختلافات معنوية في معدلات النمو ولكن أفضل معدل بقاء سجل مع الكثافة ١١٠ يرقة/لتر. التجربة الثانية أختبرت أربع فترات إضاءة مختلفة (٦: ١٨، ١٢: ١٢، ١٨: ٦، ٢٤: ٠ ساعات إضاءة: ساعات إظلام) وأوضحت النتائج أن أفضل معدلات نمو لليرقات عند الفترة الضوئية (١٨: ٦ ، ٢٤: ٠ ساعات إضاءة: ساعات إظلام). التجربة الثالثة أختبرت خمسة أغذية مختلفة (غذاء حي (روتيفر وأرتيميا) وبيض الدجاج (البيضة الكاملة، بياض البيض، صفار البيض، صفار البيض المسلوق)) وأوضحت النتائج أن أعلى معدلات نمو سجلت عند التغذية على الغذاء الحي. وأن أفضل معدل بقاء سجل مع التغذية على الغذاء الحي وبياض البيض.