Effect of some rearing systems 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 salinity and temperature on growth and development of *Solea aegyptiaca* larvae and weaning feed type on growth of *Solea aegyptiaca* post-larvae. The first experiment was tested five different salinities (10, 15, 20, 25 and 30‰). The results found that, no significant difference in growth rate, but the best performance and survival rate of larvae was at salinity 20‰. The second experiment was tested eight different water temperature (16, 17, 18, 19, 20, 21, 22 and 23°C). The results showed that, the larvae under temperature 20 °C showed the highest growth rate, while the best survival rate was recorded at temperature 16 °C under experimental conditions. The growth rate for larvae has been increased gradually with increasing temperature until 20 °C then decreased, while survival rate was decreased gradually with increasing temperature. The third experiment was tested three different feed (*Artemia* (T_1), *Artemia* + artificial feed (T_2) and artificial feed (T_3)) of *Solea aegyptiaca* post-larvae weaning. Results of growth performance parameters were highest with post-larvae fed on (T_1) compared to the other feeds (T_2 and T_3). Larvae fed on (T_1 and T_2) led to higher survival rate compared to fed on (T_3).

Keywords: Growth performance, development, survival rate, salinity, temperature, *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; 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; Dinis and Reis, 1995); the weaning, switch from live prey to compound diet feeding, induces poor growth and mortality (Dinis, 1992; 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 (Olsen *et al.,* 2000; Olafsen, 2001). 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). Weaning seems to be more difficult in sole. In addition, the early metamorphosis and acquisition of a peculiar non-proactive bottom-feeding behavior make early weaning an important issue in sole hatcheries. Engrola *et al.* (2007) studied three different strategies of weaning trying to find out the optimum Senegalese sole weaning scheme. In other approach, Engrola *et al.* (2009) recommended co-feeding regime with inert diet from mouth opening stage in Senegalese sole, as the co-fed sole were larger and have less tail deformities.

Salinity influences energy expenditure in fish; there is a significant energetic cost associated with the mechanisms used by fish to maintain osmotic balance (Boeuf and Payan, 2001). Osmoregulatory cost is generally lowest under isosmotic conditions (Jobling, 1994; Likongwe *et al.,* 1996) and can increase when moving from stable to variable salinity environments (Hutchinson and Hawkins, 1990). However, an isosmotic environment is not preferential for all species where optimal salinity in terms of growth and condition can vary during onto-genetic development (Allen and Chec, 2007; Cardona, 2000; Partridge and Jenkins, 2002). From a review of the literature, Deacon and Hecht (1999) showed that in general, marine spawned fish grew better at salinities higher than the isosmotic level, whilst fresh water spawned fish had optimal growth below the isosmotic level. Information on species specific salinity tolerances and their interaction with ontogenetic development is useful for maximising growth rates, condition and development rates in aquaculture, whilst recognition of environmental conditions promoting enhanced growth, survival and recruitment can aid the identification of high-quality nursery habitats.

Temperature is the most important environmental parameter driving development, growth, and survival of marine fish during their early life history (Pepin, 1991). Some studies have demonstrated the existence of daily rhythms of temperature selection in fish in wild conditions. In such studies, fish showed daily migrations as they searched for a preferred temperature for physiological activity and growth (Gibson *et al.,* 1998; Sims *et al.,* 2006). In Senegalese sole, most studies on biological development and temperature have used a constant temperature of 20 ºC (Parra and Yufera, 1999; Yufera *et al.,* 1999; Canavate *et al.,* 2006).

The aim of the present study was to assess the overall effects of salinity and temperature on the growth and development of the Egyptian sole (*Solea aegyptiaca*) during early life stages. Moreover, to investigate the effect of feed type on weaning post-larvae on growth and survival rates.

MATERIALS 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% 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 periods (Mean±S.E).

Parameters	Experiments larval rearing		
	Salinity	Temperature	Weaning post-larvae
Temperature, °C	17.75±1.1	--	19.25±1.05
pH	8.29±07	8.31±0.08	8.24±0.06
Salinity, ‰	--	33±3	33±1
Dissolved oxygen, mg/l	8.55±0.35	7.7±0.7	8.5±0.5
Total ammonia, mg/l	0.72±0.04	0.62±0.16	0.53±0.08
Un-ionized ammonia, mg/l	0.037±0.002	0.035±0.017	0.031±0.004
Nitrite, mg/l	0.326±0.005	0.258±0.069	0.218±0.03
Nitrate, mg/l	1.19±0.005	1.11±0.21	1.02±0.13

Culture of live foo

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, 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 x 10^5cell/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 was 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 4g 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 were transported to the larval rearing tanks, after the start of the exogenous feeding, at a density of 80 larvae per litter. *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) (Table 2). *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 salinity

The first experiment was conducted to investigate the effect of salinity, (10, 15, 20, 25 and 30‰) on growth performance, metamorphosis stage and survival rate of *Solea aegyptiaca* larvae. Larvae of 2.72 ±0.02 mm initial mean length were randomly distributed into the experimental circular plastic tanks of 25 L water capacity by using 10 experimental plastic tanks.

Table 2: 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

The second experiment: Effect of temperature

The second experiment was conducted to investigate the effect of water temperature, (16, 17, 18, 19, 20, 21, 22 and 23°C) on growth performance, metamorphosis stage and survival rate of *Solea aegyptiaca* larvae. Larvae of 2.72 ±0.02 mm initial mean length were randomly distributed into the experimental rectangular glass aquaria of 25 L water capacity by using 16 experimental glass aquaria.

The temperature was controlled by a heater with thermostat (JAGER 3609 Aquarium Heater, Automatic heater, Germany).

The third experiment: Effect of feed type on weaning post-larvae

The fourth experiment was conducted to investigate the effect of feed type on weaning post-larvae, (*Artemia* (T_1), *Artemia* + artificial feed (T_2) and artificial feed (T_3)) on growth performance and survival rate of *Solea aegyptiaca* post-larvae. Newly hatched larvae of *Solea aegyptiaca* were reared in 25 L water capacity glass aquaria and plastic tanks, from 4 DAH to metamorphosis stage, and then they were transported to the weaning aquaria until the end of the experiment. The weaning trail, were carried out in rectangular glass aquaria containing 25 liters of saline water. For each glass aquarium aeration was adjusted and provided by sand layer of 2 cm in the bottom of each aquarium. Post-larvae of 11.50 ±0.10 mg initial mean weight, were randomly distributed into the experimental aquaria of 25 L water capacity by using 6 experimental glass aquaria at density of 6 post-larvae/L. Post-larvae rearing was following up from metamorphosis stage (36 DAH) until the end of the experiment (100 days trial period, from 5/2/2015 to 16/5/2015), with a feeding regime based on artificial diet (Table 3) and live food. By the age of metamorphosis stage, it fed on *Artemia* metanauplii (8-12 ind./ml). Feeding regime post-larvae were fed 4 liter *Artemia*/ T1, 2 liter *Artemia* + 1g artificial diet/ T2 and 2g artificial diet/ T3 per day.

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.
- Weight gain (mg) = final weight, mg - initial weight, mg.
- Average daily weight gain (mg) = average weight gain, mg/ experimental period, day.
- Specific growth rate (SGR, %) = [(ln final weight -ln initial weight)/ period in days] × 100, where ln is the natural log.
- 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.

Table 3: Ingredients and chemical composition of the diet used in the weaning post-larvae experiment.

Ingredients, %	Weaning diet	Chemical composition	Weaning diet
Fish meal, (CP 72%)	70	Dry matter, DM %	91.24
Starch	22	Crude protein, CP %	50.40
Fish oil	3	Ether extract, EE %	14.00
Sunflower oil	4	Crude fiber, CF %	0.00
vit & Min. *	1	Ash, %	7.09
- - - - - - -	- -	Nitrogen free extract, NFE % **	19.75
- - - - - - -	- -	GE, kcal/g***	4.853

* Vitamins and minerals mixture each 3 Kg of mixture contains: 12000 00 IU Vit. A, 3000 00 IU Vit. D3, 700 mg Vit. E, 500 mg Vit. K3, 500 mg Vit. B1, 200 mg Vit. B2, 600 mg Vit. B6, 3 mg Vit. B12, 450 mg Vit. C, 3000 mg Niacin, 3000 mg Methionine, 10000 mg Cholin chloride, 300 mg Folic acid, 6 mg Biotin, 670 mg Panthonic acid, 3000 mg Magnesium sulphat, 3000 mg Copper sulphat, 10000 mg Iron sulphat, 1800 mg Zinc sulphat, 300 mg Cobalt sulphat.

** Calculated by differences . *** Calculated according to NRC, 1993.

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 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 completed 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.

Larval development stages and metamorphosis stage

There are five distinct line phases. These, can be related to the five different developmental stages of the larvae and post larvae. The first phase of wholly endogenous nutrition lasted for 3 days in all trials. At the first DAH, newly hatched larvae, symmetrical larvae with yolk sac and its tail flexure was retained, and the larvae swam in straight direction. The length of larvae was about 1.42 mm. The yolk sac diameter was 1.0x0.68 mm which contains oil globules ranged from 0.084 to 0.1 mm. The eyes are not pigmented. The mouth not open and the larvae were endogenous feeding; it depends on the nutrients storage on the yolk sac. This was characterized by an initial rapid length increase prior to mouth opening and first feeding.

The second phase of transition from endogenous to exogenous nutrition occurred from 3-6 DAH. At age about 4 DAH, the total length was between 2.70 to 2.75 mm. The mouth was opened and its jaws were developed. The eyes were more pigmented comparing to the previous stage. The yolk sac was reduced to less than one third its initial size (0.20x0.22 mm). It was characterized by a retarded increase in length that coincided with depletion of yolk sac reserves. The third phase of accelerated growth occurred from about 8 DAH onward in fed larvae, and nutrition was entirely exogenous. The fourth stage of formation of caudal fin rays and ventral and dorsal fin rays extends from 10 DAH. The fifth, start of metamorphosis from 9-17 days and continued until metamorphosis is

completed when the left eye transferred to right side of the head at about 16-36 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 16-36 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 (Fig.1). Metamorphosis was different according to the variability in salinity and temperature in the following trials larvae rearing.

Fig. 1: Developmental stages of the Egyptian sole, *Solea aegyptiaca* larvae.
A). The fertilized egg, with the yolk sac (YS) and oil globules (OG).
B). The newly hatching larvae at the first DAH. It shows, eye is not pigmented (E). The mouth is not opened (M). The yolk sac (YS), with oil globules (OG), is very abundant and feeding is exclusively endogenous.
C). The larva at 4 (DAH). It shows, eyes are nearly pigmented (E). The mouth is opened (M). Larvae are feeding exogenous but some yolk sac (YS) is still remains.
D). The post-larvae at metamorphosis stage. It shows, dorsal fin (DF), caudal fin (CF) and anal fin (AF). Both eyes are on the right side of the body and the sole assumes the benthic behavior typical flatfish.

Behavior of larvae

Newly hatched larvae were buoyant at hatching for about 3-4 days. This is due to the presence of the yolk sac which contains oil globules. These larvae

were grouped in the surface water layer and collected themselves in one location. After complete resorption of yolk sac and formation of pectoral fin on each sides of the trunk region of larvae that were easily swim up and down, and appear to be very flexible, bending their bodies in certain movement. At 15 DAH, the larvae started sinking to the bottom and sometimes, larvae may attach themselves on wall, also their flexibility began to decrease and lose. The larva was developed to complete metamorphosis and they were considered as bottom feeder and started to burrow into the sand.

The first experiment: Effect of salinity on growth performance, metamorphosis stage and survival rate

Results of growth performance, metamorphosis stage and survival rate for larvae reared in different salinity levels are present in Table (4). Survival rate values were relatively highest with larvae reared in 20‰, while the larvae under 30‰ showed a lower survival rate, with significant differences (P≤0.05) were observed. The results showed that insignificant differences (P≤0.05) were obtained in all growth performance parameters between treatments. At the end of the trial (end of metamorphosis), average final weight and final length of larvae were 10.85, 9.55, 10.85, 10.80, 10.05 mg and 10.05, 9.20, 10.50, 10.50, 9.50 mm, for larvae reared in salinity 10, 15, 20, 25 and 30‰, respectively. Metamorphosis of the sampled speciemens were completed at 36.5, 34.5, 32.5, 34.0, 36.0 DAH for salinities 10, 15, 20, 25 and 30‰, respectively. The results indicated that salinity (20‰) improved the growth rate of larvae without any statistical difference between treatments (10, 15, 20, 25 and 30‰) under experimental conditions.

Table 4: Effect of salinity on growth performance, metamorphosis stage and survival rate of the Egyptian sole, *Solea aegyptiaca*, larvae.

Items	Salinity					SED*
	10 ‰	15 ‰	20 ‰	25 ‰	30 ‰	
Initial aveg. Length, mm/larvae	2.70	2.74	2.72	2.70	2.73	0.017
Final aveg. Length, mm/larvae	10.05	9.20	10.50	10.50	9.50	1.057
Total length gain, mm/larvae	7.35	6.47	7.78	7.80	6.77	1.065
Average daily length gain, mm/ larvae/ day	0.21	0.20	0.24	0.23	0.20	0.021
Final aveg. Weight, mg/larvae	10.85	9.55	10.85	10.80	10.05	1.886
Condition factor, mg/mm^3	1.07	1.23	0.94	0.95	1.17	0.145
Metamorphosis stage, day	36.5	34.5	32.5	34.0	36.0	2.258
Survival rate, %	11.88^{b}	11.75^{bc}	12.80^{a}	11.25^{cd}	10.70^{d}	0.229

- (a, b, c) 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 temperature on growth performance, metamorphosis stage and survival rate

Results of growth performance, metamorphosis stage and survival rate for larvae reared in different water temperature are present in Table (5). Survival rate values were relatively highest with larvae reared in 16 °C, while the larvae under temperature (22°C and 23°C) showed a lower survival rate, with significant differences (P≤0.05) were observed. 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 different temperature showed differences in weight and length. The larvae under temperature 20 °C showed a highest growth rate during the experiment (which reached 14.50 mm in length, 32.85 mg in weight, complete metamorphosis at 22 DAH), while the larvae under temperature 16 °C showed a lower growth rate during the experiment (which reached

9.50 mm in length, 11.25 mg in weight, complete metamorphosis at 36 DAH). Significant differences at the end of metamorphosis were detected among treatments. The metamorphosis of group which reared under temperature 21, 22 and 23 °C was the most fast in the other temperature, while larvae reared under temperature 16 and 17°C were the most slower in the other temperature. These results indicated that the best growth rate for larvae was obtained at temperature 20°C, while the best survival rate was recorded at temperature 16 °C under experimental conditions. The growth rate for larvae has been increased gradually with increasing temperature until 20°C then decreased. Survival rate decreased gradually with increasing temperature.

Table 5: Effect of temperature on growth performance, metamorphosis stage and survival rate of the Egyptian sole, *Solea aegyptiaca*, larvae.

Items	Temperature, °C								SED*
	16°C	17°C	18°C	19°C	20°C	21°C	22°C	23°C	
IL	2.72	2.72	2.73	2.74	2.72	2.74	2.70	2.74	0.032
FL	9.50^{b}	10.50^{ab}	10.90^{ab}	11.20^{ab}	14.50^{a}	11.50^{ab}	10.50^{ab}	10.50^{ab}	1.489
TLG	6.79^{b}	7.79^{ab}	8.17^{ab}	8.47^{ab}	11.78^{a}	8.77^{ab}	7.80^{ab}	7.77^{ab}	1.471
ADL	0.19^{d}	0.22^{cd}	0.31^{cd}	0.38^{bc}	0.54^{ab}	0.57^{a}	0.49^{ab}	0.51^{ab}	0.070
FW	11.25^{b}	14.05^{b}	15.00^{b}	16.65^{b}	32.85^{a}	17.75^{b}	11.50^{b}	11.00^{b}	4.268
K	1.31	1.24	1.15	1.19	1.09	1.16	0.99	0.96	0.187
Meta.	36.0^{a}	36.5^{a}	26.5^{b}	22.0^{c}	22.0^{c}	15.5^{d}	16.0^{d}	15.5^{d}	1.118
SR,%	11.75^{a}	10.65^{b}	8.55^{c}	7.43^{d}	7.40^{d}	7.13^{d}	7.08^{d}	7.05^{d}	0.372

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

IL- Initial aveg. Length, mm/larvae, Fl- Final aveg. Length, mm/larvae, TLG-Total length gain, mm/larvae, ADL- Average daily length gain, mm/ larvae/ day, FW- Final aveg. Weight, mg/larvae, K- Condition factor, mg/mm^3, Meta.- Metamorphosis stage, day, SR,% - Survival rate, %

The third experiment: Effect of feed type on growth performance and survival rate of weaning post-larvae

Results of growth performance parameters and survival rate of *Solea aegyptiaca* post-larvae fed on the different feed are shown in Table (7). There was no significant difference in the initial average length and weight of the post-larvae among treatments.

Table 7: Effect of feed type on growth performance and survival rate of weaning post-larvae of the Egyptian sole, *Solea aegyptiaca*.

Items	Feed type			SED*
	T_1	T_2	T_3	
Initial aveg. Length, mm/larvae	10.50	10.50	10.00	1.527
Final aveg. Length, mm/larvae	47.50^{a}	35.50^{b}	25.00^{c}	3.317
Initial aveg. Weight, mg/fish	11.50	11.60	11.30	0.757
Final aveg. Weight, mg/fish	663.50^{a}	255.00^{b}	111.50^{c}	25.357
Total weight gain, mg/fish	652.00^{a}	243.40^{b}	100.20^{c}	24.774
Average daily weight gain, mg/fish /day	6.52^{a}	2.43^{b}	1.00^{c}	0.247
Specific growth rate (SGR), %/day	4.06^{a}	3.09^{b}	2.26^{c}	0.182
Condition factor, mg/mm^3	0.62	0.60	0.70	0.130
Survival rate, %	76.93^{a}	73.08^{a}	34.62^{b}	7.693

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

Post-larvae fed on different feeds showed significant differences (P≤0.05) in survival rate between treatments; post-larvae fed on (*Artemia* (T_1) and *Artemia* + artificial feed (T_2)) led to higher survival compared to fed on artificial feed (T_3).

The results showed that significant differences (P≤0.05) were obtained in all growth performance parameters between treatments, except the condition factor. Results of growth performance parameters were highest with fed on T_1 compared to the other feeds (T_2 and T_3). Results show that final length (mm), final weight (mg), SGR (%) and survival rates (%) for feeding on T_1, T_2 and T_3 were (47.50, 35.50, 25.00 mm), (663.50, 255.00, 111.50 mg), (4.06, 3.09, 2.26 %) and (76.93, 73.08, 34.62 %), respectively. These results indicated that the best growth rate for post-larvae was obtained at feeding on *Artemia* then feeding on *Artemia* + artificial feed under experimental conditions. Post-larvae fed *Artemia* showed statistically higher growth comparing to all the other groups.

DISCUSSION

Generally, in the present trials the larval survival rates (7-12.80%) 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 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.

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 is 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; Daniels *et al.,* 1996).

Effect of salinity

The possibility of successfully rearing sole larvae under reduced salinity conditions would lead to new options for hatchery facilities where only brackish water is available. In the present study, the following five salinities (10, 15, 20, 25 and 30‰) were tested on *Solea aegyptiaca* larvae and found no significant difference in growth, but the best performance of larvae was at salinity 20‰. These results similar to that of Salas-Leiton *et al.* (2012) who reported that larval rearing until complete metamorphosis under standard culture conditions demonstrated that *Solea senegalensis* larvae could be successfully reared at 10‰ if transference to

this low salinity is carried out once the mouth opening process has been completed (2–3 DAH). They added no significant differences were found between salinities of 10 and 33‰ when feeding activity was analysed in the first six rearing days. Both salinities led to similar individual dry weights throughout the experimental period, with significantly higher weights recorded in larvae grown at 10‰ salinity only at 21 DAH. With the exception of 14 DAH, the higher weight achieved at 21 DAH in our larvae reared at 10‰ salinity is probably associated with decreased survival, and consequently with lower stocking densities during the last days of metamorphosis (Salas-Leiton *et al.,* 2012). Also, similar results in the flatfish European flounder (*Platichthys flesus*) were obtained by O'Neill *et al.* (2011) who found that, no significant difference in ontogenetic development between exposures (salinity of 0, 10, 20 and 30‰). No significant differences in somatic growth rate, somatic condition or standard length were observed between treatments.

The relationship between salinity and growth rate throughout the larval stages in the flatfish, both Brazilian and greenback flounders showed optimum growth over salinities ranging from 20 to 30‰ (Sampaio *et al.,* 2007) and from 15 to 35‰, respectively (Hart *et al.,* 1996). The spotted halibut (*Verasper variegatus*) preferred moderately low salinities (8–16‰) during the larva–juvenile transformation period (Wada *et al.,* 2004). Turbot inhabiting the North Sea had optimum rates at salinities above 20‰ (Karas and Klingsheim, 1997).

The results of the current study, showed that under experimental conditions of salinity, there were insignificant differences between treatments in the development of metamorphosis, a process completed 32.5-36.5 DAH under different salinities. Similar results were obtained by Salas-Leiton *et al.* (2012) who showed that, salinity did not induce differences in the development of metamorphosis between 10 and 33‰ salinity, but a process completed 21 DAH under both salinities for *Solea senegalensis*.

In this study, survival rate values were relatively highest with larvae reared in 20‰, while the larvae under 30‰ showed a lower survival rate, with significant differences between different salinities. Similarly, Moustakas *et al.,* (2004) found that, larvae of southern flounder (*Paralichtys lethostigma*) showed reduced survival and markedly lower growth rates at full-strength seawater (35‰) compared to that achieved at a lower salinity (25‰) from hatching to 15 DAH. Also, Kerstan (1991) found that within estuaries, densities of juvenile *P. flesus* significantly increased with decreasing salinity. On the other hand, a higher final survival rate was obtained in *Solea senegalensis* larvae cultivated at 33‰ compared to 10‰ salinity (Salas-Leiton *et al.,* 2012). Also, lower survival rates were obtained in greenback flounder larvae reared at 15‰ salinity in comparison to either 25 or 35‰ (Hart *et al.,* 1996). Moreover, Salas-Leiton *et al.* (2012) found that, newly hatched and early-developing yolk sac larvae presented similar survival rates 3 DAH when exposed to salinities of 10, 18, 27 and 33‰, one noticeable finding was the diverse jaw abnormalities (affecting 100% of larvae) and the subsequent ingestion inability associated with a salinity of 10‰.

The results of the present study indicated that, *Solea aegyptiaca* might be successfully developed until metamorphosis over the range 10–30‰, and the ability of *Solea aegyptiaca* larvae to grow over a wide salinity range previously. Our overall results demonstrated that *Solea aegyptiaca* larvae have the capacity to be successfully reared following standard procedures at salinities as low as 10‰, with the only requisite being completed mouth development before transference to

low salinity. That is, larvae can be kept in incubators at medium or high salinity (≥18‰) until 2 DAH and then transferred to rearing tanks recommended by Salas-Leiton *et al.* (2012) for *Solea senegalensis*. Spontaneous activity and swimming behaviour (Boeuf and Payan, 2001) as well as food consumption, digestion and absorption of prey can be altered under different salinity regimes (Jobling, 1994; Boeuf and Payan, 2001). These processes can affect energy expenditure and consequentey fish condition (O'Neill *et al.,* 2011).

Effect of temperature

Temperature represents the most important environmental factor that drive development, growth, and survival of marine fish during their early life history (Pepin, 1991). In the present study, eight different temperatures (16, 17, 18, 19, 20, 21, 22 and 23°C) were tested for *Solea aegyptiaca* larvae. The larvae under temperature 20 °C showed the highest growth rate, while the larvae under temperature 16 °C showed the lower growth rate during the experiment. The growth rate for larvae has been increased gradually with increasing temperature until 20 °C then decreased. These results agreed with most studies on biological development and temperature in Senegal sole as many authors have used a constant temperature of 20 ºC (Martinez *et al.,* 1999; Parra and Yufera, 1999; Ribeiro *et al.,* 1999; Yufera *et al.,* 1999; Canavate *et al.,* 2006; Sanchez *et al.,* 2010; Salas-Leiton *et al.*, 2011, 2012). The Senegal sole (*Solea senegalensis*) is a flatfish adapted to temperate waters of around 20-21 ºC (Drake *et al.,* 1984).

On the other hand, Blanco-Vives *et al.* (2010) reported that, *Solea senegalensis* larvae were exposed to constant temperature (20.7 °C), 22.1 °C day: 19.0 °C night and 19.2 °C day: 22.0 °C night. The sole larvae achieved the best growth performance, and fastest development under thermocycle conditions (22.1 °C day: 19.0 °C night). Larvae under (19 °C day/22 °C night) showed lower growth than those under the other treatments. In addition, Palazzi *et al.* (2006) recorded that, growth rate of *S. solea* reached the maximum incremental rate between 20 and 25°C. Also, Irvin (1973) studied rearing of *S. solea* juvenile at five temperatures ranging from 11 to 27 ºC for 12 weeks. The fish showed an approximately linear increase in growth from 9 to 23 ºC and a drop in growth after that. Fonds (1976) studied also rearing of *S. solea* juvenile at temperatures ranging from 10 to 25 ºC for over a year. In his study he found that the fish grew slower as they were larger than the fish in Irvin's (1973) study, but both experiments showed little increase in growth rates above 20 ºC and indicated that the optimum temperature for growth was between 20 and 25 ºC. Yolk utilization efficiency decreases towards the limits of a species range of temperature tolerance (Heming and Buddington, 1988). Development of sole eggs succeed at temperature ranged from 7 to 19 °C, while, larval growth of sole was lower than that ranged from 10 to 23 °C (Fonds, 1979).

The results of the current study, showed a significant differences in completion of metamorphosis at 36, 36.5, 26.5, 22, 22, 15.5, 16 and 15.5 DAH for temperature 16, 17, 18, 19, 20, 21, 22 and 23°C, respectively. Similar results were obtained by Zaki *et al.* (1998) showed that, metamorphosis of *Solea aegyptiaca* was completed at 18 DAH at 21.5 ºC. Also, similar results on *Solea solea* were reported by Palazzi *et al.* (2006) and Lund *et al.* (2007) showed that, metamorphosis was completed at 25 DAH at 18 ºC. Also, Blanco-Vives *et al.* (2010) showed that, metamorphosis of *Solea senegalensis* was completed at 17 DAH at (22.1 °C day: 19.0 °C night). Larvae reared under constant temperature (20.7 °C) completed the metamorphosis at 17 DAH, while larvae exposed to (19 °C day/22 °C night) completed at 19 DAH. Contrarily, Zaki *et al.* (1998) showed that,

metamorphosis of *Solea aegyptiaca* was completed at 27 and 29 DAH at 20 and 15 ºC, respectively. Also, Bonaldo *et al*. (2011) showed that, metamorphosis of *Solea solea* was completed at 33 DAH at 18 ºC. Also, Dinis *et al*. (1996, 1999) showed that, metamorphosis of *S. senegalensis* was completed at 19 DAH at 18 ºC. As well as, Yufera *et al.* (1999) reared larvae of *S. senegalensis* until the end of metamorphosis at 17 DAH at 19.5ºC.

In this study, survival rate values were relatively highest with larvae reared in 16 °C, while the larvae under temperature (22°C and 23°C) showed a lower survival rate. Survival rate decreased gradually with increasing temperature. Similar results on *Solea aegyptiaca* were reported by Zaki *et al*. (1998) as survival rate through embryonic developmental stages were 83.3% at 15 °C, 83% at 16 °C, 82% at 20 °C, survival rate was little decreased with increasing temperature. Also, Fonds (1979) incubated sole eggs at five different temperatures between 10 and 22 ºC, high survival and normal development until hatching were observed at temperatures from 10 to 16 ºC. On the other hand, the results disagree with data obtained by Blanco-Vives *et al.* (2010) reported that, *Solea senegalensis* larvae were exposed to constant temperature (20.7 °C), 22.1 °C day: 19.0 °C night and 19.2 °C day: 22.0 °C night. The sole larvae achieved the best survival under thermocycle conditions (22.1 °C day: 19.0 °C night).

Temperature has long been reported to influence the growth, survival and development of fish larvae both in the wild and laboratory (Methot and Kramer, 1979; Pepin, 1991; Green and Fisher, 2004; Johnston *et al.,* 2004). In flatfish larvae the environmental factors may be crucial in determining recruitment to nursery grounds via their effects on growth and mortality during the metamorphosis-settlement period (Yamashita *et al.,* 2001). Some studies have demonstrated the existence of daily rhythms of temperature selection in fish in wild conditions. In such studies, fish showed daily migrations as they searched for a preferred temperature for physiological activity and growth (Gibson *et al.,* 1998; Sims *et al.,* 2006). Ottesen and Bolla (1998) reported that some physical parameters, including inappropriate temperature or salinity; may be associated with jaw malformation due to mechanical damage in cultured larvae. The temperature in water undergoes changes according to the climatic fluctuations (Chapman and Kimstach, 1996). Surface water temperatures are affected by many different factors; season, altitude, latitude, time of day, cloud cover, air circulation and depth and flow of the body of water. Furthermore, fish are more susceptible to disease at extreme temperatures (Holt *et al.,* 1975). Provided that food availability is unrestricted, metabolic rate increases as temperature increases. Growth is found to decrease at temperatures above the optimum because of a possible decrease in appetite and high energy cost of maintenance metabolism (Jobling, 1994). At low temperatures, growth is restricted because of low metabolic rates and low food intake. Hence, when culturing a species, best performance is obtained when temperatures are optimal.

Effect of feed type on weaning post-larvae

Weaning and metamorphosis which are accompanied by increased mortality rates (Rueda-Jasso *et al.,* 2005). The utilization of a co-feeding regimen, which gradually weans larvae off live preys has been able to promote digestive maturation at early age (Engrola *et al.,* 2007, 2009) and to improve growth performances and survival rate of marine fish larvae (Rosenlund *et al.,* 1997). A precocious substitution of live feed with micro-diets could promote the early adaptation of larvae to dry feed (Gatesoupe and Luquet, 1982). In previous studies on sole weaning, weaning was considered the major obstacle to sole rearing and several attempts were made to obtain suitable weaning diets (Bromley,

1977; Metailler *et al.,* 1981; Appelbaum, 1985; Canavate and Fernandez-Diaz, 1999), focusing on the chemical substances acting as feeding stimulants (Mackie *et al.,* 1980; Cadena-Roa *et al.,* 1982; Metailler *et al.,* 1983). Diet palatability and digestibility both appear to be critical aspects in sole weaning. According to Gatesoupe and Luquet (1982) there is a choice to be made between two ways of weaning (i.e., *Artemia* for 25 days (Fuchs, 1978) or *Artemia* for 5–15 days supplemented with an artificial diet (Gatesoupe and Luquet, 1982). The first one gives higher growth rate but also high costs (due do *Artemia* expenses), and the second alternative is less costly but yields lower growth. In the study conducted by Villalta *et al.* (2005), larvae of Senegal sole fed on the diet containing the highest docosaesaenoic acid (DHA) concentration, had the lowest growth in length comparing to the other groups.

In the present study, three different feed: (*Artemia, Artemia* + artificial feed and artificial feed) of weaning *Solea aegyptiaca* post-larvae were tested. Results of growth performance parameters were highest with post-larvae fed on *Artemia* compared to the other feeds (*Artemia* + artificial feed and artificial feed). Larvae fed on (*Artemia* and *Artemia* + artificial feed) led to higher survival compared to that fed on artificial feed. These results agreed with the findings of Bonaldo *et al.* (2011) who tested different weaning strategies on *Solea solea* using the commercial micro-diets, they obtained a very early weaning using commercial micro-diets in common sole larvae affects growth performance but no statistical differences among groups were observed in survival rates with better survival rate in control (fed on *Artemia*). Also, Koven *et al.* (2001) reported that, the poor performance of Sea bream larvae exclusively fed on micro-diets is related to the variable acceptance and attraction of the inert particle compounded by inadequate ingestion, digestion and assimilation. Physico-chemical characteristics of micro-diets such as color, shape, particle size, sedimentation rate or release of attractants can influence feed intake by very young fish (Saenz de Rodriganez *et al.,* 2011). In addition, larvae have to be able to digest the particles and be able to absorb and assimilate the nutrients that must fit the requirement of the larvae (Kvale *et al.,* 2006).

On the other hand, Palazzi *et al.* (2006) Carried out, three weaning trials for *Solea solea* comparing two commercial feeds on larvae about 30 DAH. One of these feeds was sufficient in itself to complete juvenile weaning, reaching average survival rates of 85%, which are comparable to those obtained in the control groups fed on live *Artemia*. Average survival rates of 43% were obtained with the second commercial feed. Both commercial feeds enabled superior juvenile growth on average to that in the control groups. Also, Engrola *et al.* (2007) tested different weaning strategies on Senegal sole (*Solea senegalensis*) using the commercial micro-diets. They obtained that survival rates were not exceeding 40% at 26 and 33 DAH, but up to 90% when larvae were weaned at 40 and 60 DAH. In addition, studies (Howell, 1998; Day *et al*., 1999) have demonstrated that young sole can be weaned on to commercially prepared formulated feeds with high survival and growth approaching those attainable on live foods.

In the present study, *Solea aegyptiaca* larvae fed on *Artemia* led to higher growth and survival compared to fed on artificial feed only might be related to the following: Live feeds (as *Artemia*) are able to swim in the water column and are thus constantly available to the larvae. Formulated diets tend to aggregate on the water surface or, more commonly, sink quickly to the bottom, and are thus normally less available to the larvae than are the live feeds. 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. Formulated diets 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 taken into the mouth, compared to the hard, dry formulated diets. Any food 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, the best performance and survival rate of larvae is at salinity 20‰. The larvae under water temperature 20°C showed a highest growth rate, where growth has been increased gradually with increasing temperature until 20°C then decreased, while survival rate decreased gradually with increasing temperature. The best feed for post-larvae weaning is *Artemia* only under experimental conditions. So, it is recommended to *Solea aegyptiaca* larval rearing under salinity (20‰). Reared of larvae under temperature 16-17°C from 1 DAH to metamorphosis stage, because of its high survival rate at this temperature, then rearing under temperature 20°C from metamorphosis stage to onward, because of its high growth rate at this temperature, and feeding on *Artemia* for post-larvae weaning.

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ARABIC SUMMARY

تأثير بعض نظم الرعاية على نمو وتطور يرقات أسماك موسى المصرية

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أجريت هذه الدراسة لتقييم تأثير كل من درجة الملوحة والحرارة على نمو وتطور يرقات أسماك موسى المصرية (*Solea aegyptiaca*) ونوع غذاء الفطام على النمو. التجربة الأولى أختبرت خمس درجات ملوحة مختلفة (١٠، ١٥، ٢٠، ٢٥ و ٣٠ جزء في الألف) حيث النتائج أنه لا يوجد اختلافات معنوية في معدل النمو ولكن أفضل أداء للنمو ومعدل البقاء لليرقات كان عند ملوحة ٢٠ جزء في الألف. التجربة الثانية أختبرت ثماني درجات حرارة مختلفة (١٦، ١٧، ١٨، ١٩، ٢٠، ٢١، ٢٢ و ٢٣ م°) حيث النتائج أن اليرقات المعرضة لدرجة حرارة ٢٠ سجلت أعلى معدلات نمو بينما أفضل معدل بقاء سجل عند درجة حرارة ١٦ م° وأن نمو اليرقات كان يزداد تدريجيا مع زيادة درجة الحرارة حتى ٢٠ م° بعد ذلك بينما مع معدل البقاء كان ينقص تدريجيا مع زيادة الحرارة. التجربة الثالثة أختبرت ثلاثة أغذية للفطام مختلفة (أرتيميا، أرتيميا + غذاء صناعي وغذاء صناعي) وأوضحت النتائج أن أعلى معدلات نمو سجلت عند التغذية على الأرتيميا فقط.