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The impact of live food enrichment on the growth performance and survival rate of thin-lipped mullet, *Liza ramada* larvae

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ABSTRACT

The feeding success of *Liza ramada* larvae at early developmental stages depends on the provision of suitable food and rearing environment and on the visibility and adequate density of the prey. In the present study, the suitability of the marine enriched rotifers, *Brachionus plicatilis* as a starter food followed by enriched *Artemia* nauplii and their effects on the growth performanceand survival ratewere investigated for rearing of *Liza ramada* larvae.

Induced spawning of mature *Liza ramada* breeders was done to produce the larvae. After hatching, the larvae were stocked at a density of 20 larvae/l in 50-l glass aquaria, under different feeding conditions for three periods; green water conditions; with *Nannochloropsis oculata* (2.5×10^5 cells/ml) for 7 days, rotifers (8–10 individuals/ml) for 28 days and *Artemia salina*nuplii (4–6 individuals/ml) for 28 days. Four groups were applied for both rotifers and *Artemia*; green water conditions with rotifers or *Artemia* enriched with *N. oculata*or yeast and clear water conditions with rotifers or *Artemia* enriched with *N. oculata* or yeast.

The results showed improvement in growth and survival rates of *Liza ramada* larvae fed on green water with *Brachionus plicatilis* or *Artemia salina* enriched with *N. oculata* at the end of 7thday, 35thday and 63rdday post-hatching, respectively. This indicates that the algae *N. oculata* is essential not only for feeding requirement of larvae at first feeding but also for water quality improvement. Larval wet weight varied significantly between the treatments. However, the final length of the larvae did not vary significantly between the treatments.

In conclusion, the presence of green algae *Nannochloropsis oculata*during *Liza ramada* larval rearing, in water or as enrichment media for live food, is essential not only for growth performance but also for raising survival rate.

INTRODUCTION

The recent rapid growth of marine fish culture has been due in part to being able to overcome the bottleneck of seed production. Culture of mullets is one of the oldest forms of aquaculture, but it restricted to areas of Egypt where seed could be collected from the wild and distributed.

Its production is still remaining restricted until the induced spawning techniques are developed. This allows these valuable aquaculture species to become available worldwide.Like females of many commercially important fishes, mullets fail to complete ovarian development and do not undergo final oocyte maturation (FOM), ovulation or spawning when reared in captivity (Mousa, 1994; Mousa and Mousa, 1997; Mousa and El-Gamal, 1999 and Mousa, 2010). To facilitate a steady supply of seeds, oocyte maturation and ovulation need to be induced.

Reproduction in fish is regulated by external environmental factors that trigger internal mechanisms into action (Rottmann *et al*., 1991). The reproductive cycle can be controlled by either placing the fish in an appropriate environment or by changing the fish internal regulating factors with injected hormones or other substances (Mousa, 2010; Das, 2011 and Das *et al*., 2013). The use of hormone treatments influences ovulation synchronization variously in different fish species. The reproduction of many fish species in hatcheries is impossible without the application of hormonal preparations. This refers to spawners originating from wild fish populations (Kucharczyk *et al*., 1997 and Szabo *et al*., 2002), as well as from those reared pond conditions (Brzuska and Adamek, 2008 and Mousa, 2010). Most marine fish eggs are pelagic (Blaxter, 1969), but in salinities below a certain threshold, these eggs sink. The salinity threshold for buoyancy is important when the lower salinity tolerance of pelagic fish eggs is being considered, because sinking eggs will encounter different environmental conditions, which may or may not be conducive to embryonic (May, 1974).

The thin-lipped grey mullet, *Liza ramada* (Risso) is an important and attractive species for farming in the sea, brackish and fresh water. The culture of mullet is closely dependent on the availability of their fry. The present fry collection stations from nature can not satisfy the increasing demand for the juveniles of *Liza ramada*. Consequently, man must help the nature in such respects through developing and creating other efficient means. In other words, it is necessary to develop and establish practical techniques for artificial propagation of mullet in order to substitute the fry collection from wild stocks (Mousa, 2010).

The need to provide adequate dietary levels of essential fatty acids (EFA) for early developing fish with unusually high growth rates and large developmental demands is undoubtedly one of the highest priorities in larval rearing.A common practice for rearing marine fish larvae in captivity is to feed rotifers for several days or weeks gradually switching them over to *Artemia* nauplii and finally weaning them onto a dry feed. As rotifers and *Artemia* are naturally deficient in HUFAs, it is necessary to enrich these live feeds with essential fatty acids prior to offering them to the larvae (Conceição*et al*., 2010 and Watanabe *et al*., 2016). Enrichments frequently employed to enhance the fatty acid composition of prey items include live microalgae, microalgae paste and lipid emulsions that include various marine fish and/or PUFA oils. Furthermore, microalgae are often added to the rearing tanks along with the live prey and have been shown to enhance larval growth and survival (Naas *et al*., 1992; Faulk and Holt, 2005 and Thépot *et al*., 2016).

Therefore, the present study was aimed to determine if enriching rotifers and *Artemia* with microalgae or yeast would improve growth performance and survival rate of thin-lipped grey mullet, *Liza ramada* larvaeover the early rearing period of 63 days. In addition, the benefits of adding live algae to the larval rearing tanks were evaluated.

MATERIALS AND METHODS

Spawning and obtaining of *Liza ramada* larvae:

Broodstock collection:

Mature breeders of thin-lipped grey mullet, *Liza ramada*at least two-years-old, with total weights ranged from 300 to 600 g with total lengths 30 - 40 cm, were collected alive, by draining water completely, during the spawning season (November to January) from freshwater culture ponds (at El-Serw Fish Research Station). Sexes were identified based on spermiation for males and slightly distended abdominal condition for females. 10 females and 30 males selected from the captured fish were used in the induction spawning trials.

Seawater acclimation and induction of spawning:

The present experiments were carried out, during the natural spawning season of thin-lipped grey mullet, *Liza ramada*. The fish were anesthetized in a solution (40 mg/l) of clove oil (Sigma) before handling (Mousa, 2004). Mature females of thin-lipped grey mulletwere selected on the basis of the presence of a soft, swollen abdomen and protruding genital papillae. The maturity and the oocyte diameters of the females were staged by obtaining *in vivo* biopsy of the ovary using a polyethylene cannula (Shehadeh*et al*., 1973). The females that were used possessed oocytes whose diameters were greater than 600 µm. The diameter of at least 25 eggs of the largest oocytes was recorded from each fish, and the position of the germinal vesicle (GV) was determined after clearing the cytoplasm for 10 min with a 1:1:1 v/v methanol:ethanol:acetic acid solution (Crim and Glebe, 1990). Ripe males, in which milt could be easily extruded, by gentle pressure on their bellies, were used. Selected breeders were acclimated in 2000-litre circular fiberglass tanks (10 fish/tank). In brief, fish were transferred to water with 10‰ salinity (for 12 h) which gradually increased to 35‰ (for another 12 h).

Acclimated breeders were transferred to 500-litre fiberglass tanks equipped with constant running ozonated seawater (35‰) and aeration (Female + 2 males/tank) for induction of spawning with human chorionic gonadotropin (HCG) “pregnyl” (Nile Co. for Pharmaceuticals, Cairo, ARE). The protocol of hormonal injection was previously described by Mousa (2010).Water temperature and salinity were 19°C and 35‰ respectively. In brief, Pregnyl was injected into the dorsal musculature of fish adjacent to the dorsal fin as a priming injection at a dose of 20,000 IU/kg body weight followed 24h later by resolving injection of 40,000 IU HCG. All males received a single injection of HCG at a dosage of 1000 IU/kg body weight.

Eggs incubation and embryonic development:

The eggs were collected from each spawning tank approximately two hours after the estimated time of spawning. Eggs were collected with PVC pipes and siphoned into a plastic circular egg-collection basket (10 l) with nylon bottom (mesh size 200 µm). Buoyant viable eggs were stocked in incubators with a volume of 10l (mesh size, 200µ); 500 eggs/l. The egg incubators were placed on water surface in 500-litre circular fiberglass tanks. The experiment was carried out at 21°C. During the experiment, the flow rate of sea water into the tanks was 25% per hour. Sea water was treated by ozone, UV light, filtered and treating with antibiotics before using it. Both penicillin (10 IU/ml) and streptomycin (0.01 mg/ml) were daily added and effectively reduced bacterial growths. Experiments took place in natural photoperiod and were triplicate.

Before placing the fertilized eggs in the incubators, 30 samples were taken, measured and their mean egg diameter was calculated. Then, the eggs were

distributed among the incubators. In order to determine the common embryonic developments, 30 eggs were taken from each incubator in every thirty minutes until morula stage and then hourly intervals.

Experimental design of *Liza ramada* larvae rearing:

The newly hatched larvae were stocked at a density of 20 individuals (ind) L^{-1} in glass aquaria (50 L capacity each) containing sterilized sea water (32‰ salinity) at a temperature of 21±2°C; under different feeding conditions for three periods; clear water and green water (*Nannochloropsis oculata*, 2.5×10^5 cells/ml) for 7 days (six aquaria for each group), rotifers (8–10 ml^{-1}) for 28 days and *Artemiasalina*nauplii (4–6 ml^{-1}) for 28 days. Four groups were applied for both rotifers and *Artemia* (three aquaria for each group); green water conditions with rotifers or *Artemia* enriched with *Nannochloropsis oculata*or yeast and clear water conditions with rotifers or *Artemia* enriched with *Nannochloropsis oculata* or yeast.

The aquaria were covered with black plastic sheeting to maintain dark conditions. Rearing of larvae was carried out in static aeratedwater up to 3 days post hatching (dph). The water was partially replaced (5-6% daily) from 4 to 12 dph. Out-flowing water was strained through a 150 μm mesh. The water exchange rate was increased gradually with the age of the larvae (up to 25%). Debris and dead larvae were siphoned out each day before changing the water. The dead larvae in each aquarium were counted and recorded. Beginning 4 dph, larvae were fed with live *B. plicatilis* enriched with micro algae.

The feeding regimes for the fish larvae were initiated at 4 dph, when their mouths were opened, through 28 dph with the following sequence: feeding with the rotifer *B. plicatilis* at low density of 2-3 ind ml^{-1} on 4 dph; 3-5 individuals ml^{-1} from 5-7 dph; 8-10 individuals ml^{-1} from 8-35 dph. By 36 dph the larvae were ready to accept nauplii of *A. salina*. Thesewere fed at a density of 4-6 individuals ml^{-1} 36-63 dph. Continual addition of *N. oculata*to each fish larvae rearing aquarium was carried out to ensure enrichment for the live prey with the algae. The algae were added at a density of 100 000-250 000 cells ml^{-1} water daily to each aquarium. Microalgae also increased the oxygen content of the water and reduced the concentration of ammonia, thus serving as a water conditioner for rearing larvae.

The growth in length, weight and survival rate was recorded. Measurement of the newly hatched larvae and larval stages were made under a stereomicroscope. The measurements were taken, using an eyepiece micrometer calibrated with a standard decimal millimeter.

Measurements of growth performance:

The experiment was conducted for 63 days (January, 2016 – February, 2016). Lengths and weights of 25 randomly sampled of *Liza ramada* larvae from each treatment were weakly recorded, as well as their initial lengths and weights.Survival rate were also recorded.

Statistical Analysis

Results were expressed as mean ±S.D. Differences between treatments were tested by one-way ANOVA using the treatment as factor of variance (**Bailey, 1981)**. Tukey test was used to identify significantly different groups. Statistical significance was accepted at $P<0.05$.

RESULTS

Induced spawning and obtaining of fish larvae:

In freshwater ponds, *L. ramada* females attained to pre-spawning stage, whereas the males attained to ripe stage.Pre-spawning females contained vitellogenic oocytes varying in diameter from 600 to 650 µm. Vitellogenic (tertiary yolk) oocytes had a centrally located germinal vesicle (GV) (Fig. 1). The breeders were successfully acclimated to seawater (35‰) prior to hormonal injection. High rate (100%) of final oocyte maturation (FOM), ovulation and spawning was achieved in *L. ramada* females utilizing the pregnyl (HCG) as a priming injection at a dose of 20,000 IU/kg body weight followed, 24 h later, by resolving injection of 40,000 IU HCG/kg body weight. All injected females (10) were spawned at a time of 50 to 60 h after hormonal injection.

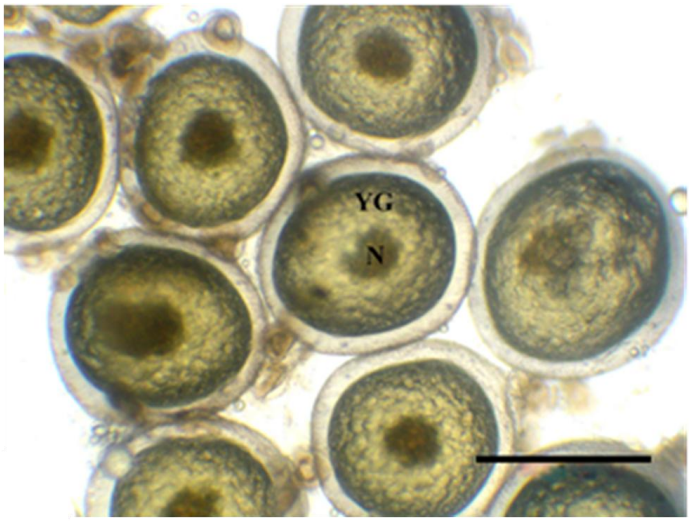

Fig. 1: Macroscopic view of pre-spawning tertiary yolk oocytes which have centrally-located nuclei (N) and yolk granules (YG). Scale bar = 500 µm.

The fertilized eggs were free, pelagic and transparent; and they varied in diameter between 0.9 and 1.0 mm. They have more than one oil globule, depending on the type and the dose of hormone; first cleavage begins at approximately 40 min. The blastodisc was situated on the bottom side of the floating egg (Fig. 2).

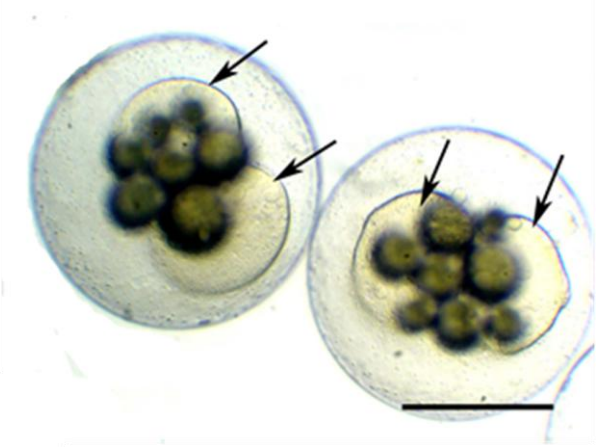

Fig. 2: Macroscopic view offertilized eggs at two-cell stage, showing that the blastodisc (arrows) was situated on the bottom side of the floating eggs. Scale bar = 500 µm.

44 h after the fertilization, lens fully formed; pigmentation get dark and various color combinations noticed (Fig. 3). The embryonic development was completed and hatching occurred at 48 h after fertilization. The body of a newly hatched larva remained curved for several hours after hatching. The larva had a large oval-shaped yolk sac with an oil droplet at its posterior end. The mouth and anus were not yet open. No pigmentation was recognized over the yolk (Fig. 4).

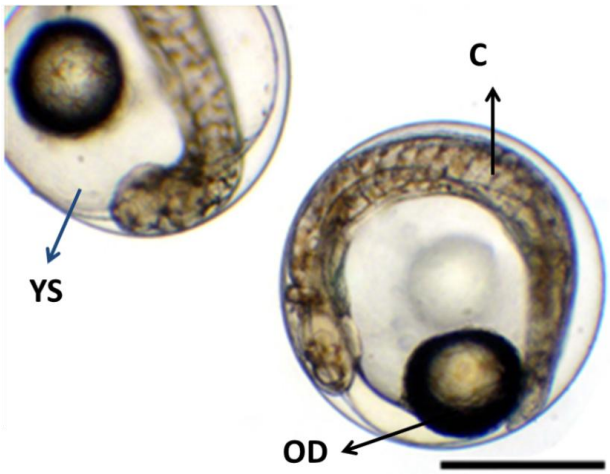

Fig. 3: Macroscopic view of developed embryoat 44 h post fertilization and showed the body is remained curved (C) and contain a large oval-shaped yolk sac (YS) with an oil droplet (OD) at its posterior end. Scale bar = 500 µm.

Fig. 4: Macroscopic view of newly hatched larva exhibiting a large oval-shaped yolk sac (YS) with an oil droplet (OD) at its posterior end. The mouth and anus were not yet open. Scale bar = 1000 µm.

Effect of feeding regimes on growth performanceof *Liza ramada* larvae:

The variations in growth performance and survival rate of *Liza ramada* larvae reared for 63 days; and fed on different feeding regimes: {green water (GW): Algae; *Nannochloropsis oculata* (N) or clear water (CW) (for 7 days); Rotifers (R) *Brachionus plicatilis*enriched with *Nannochloropsis oculata* or yeast (for 28 days) and *Artemia salina* (A)enriched with *Nannochloropsis oculata* or yeast (for 28 days)}, were given in Tables (1-3) and graphically represented in Figs. (5 - 7).

Effects of algal feeding regime on growth in length, weight and survival rate of *Liza ramada* larvae (1 - 7 dayspost-hatching):

Results in Table (1) showed that, *Liza ramada*larvae at 1-7 days after hatching,fed on different feeding regimes exhibited great variations in body length, body weight and survival rate. The mouth and anus opened on the 5th day after hatching. At the end of 7 days post-hatching, the larvae fed on green water (GW) were higher in average body length (5±0.70 mm), body weight (3±0.06 mg) and survival rate (50%) than those larvae fed on clear water (CW) (Figs. 5 - 7).

Table 1: Effects of water conditions, (for 7 days), with or without *Nannochloropsis oculata* (N), on the length (mm), weight (mg) and survival (%) at 1 - 7 days post-hatching of *Liza ramada* larvae.

Feeding regimes	**Days after hatching**				**Survival %**
Algae; *Nannochloropsis oculata* (N) (for 7 days)	**Hatching (zero day)**		**7 day**		
	L (mm)	**W (mg)**	**L (mm)**	**W (mg)**	
Green water (GW); with N	3.5±0.07	1±0.07	5±0.70	3±0.06	50 [a]
Clear water (CW); without N	3.5±0.07	1±0.07	4.5±0.33	2±0.05	30 [b]

Green water (GW); Clear water (CW); *Nannochloropsis oculata* (N).
Significantly different means ($P < 0.05$) are indicated by different letters (Tukey test).

Effects of rotifers feeding regime on growth in length, weight and survival rate of *Liza ramada* larvae (8 - 35 dayspost-hatching):

Results in Table (2) showed that, *Liza ramada* larvae at 8-35 days post-hatching, fed on different rotifers feeding regimes exhibited great variations in length, weight and survival rate. At the end of 35 days post-hatching, the highest average body length (12±0.55 mm), body weight (12±0.55 mg) and survival rate (25%) in the larvae fed on GW+R enriched with N. Lowest values in the average body length (11±0.50 mm), body weight (9.5±0.50 mg) and survival rate (19%) were recorded for the larvae fed on CW+R enriched with Y (Figs. 5 - 7).

Table 2: Effects of rotifers (R); *Brachionus plicatilis* feeding regime, with or without *Nannochloropsis oculata* (N) and yeast (Y), on the length (mm), weight (mg) and survival rate (%) at 8 - 35 days post-hatching of *Liza ramada* larvae.

Days post-hatching		**Feeding regimes Rotifers (R); *Brachionus plicatilis* (for 28 days)**			
		GW + R enriched with N	**GW + R enriched with Y**	**CW + R enriched with N**	**CW + R enriched with Y**
14	**Length (mm)**	6 ± 0.04	6 ± 0.04	5 ± 0.50	5 ± 0.50
	Weight (mg)	5 ± 0.08	5 ± 0.08	4 ± 0.50	4 ± 0.50
21	**Length (mm)**	8 ± 0.22	8 ± 0.2	7 ± 0.50	7 ± 0.50
	Weight (mg)	6 ± 0.32	6 ± 0.06	5 ± 0.45	5 ± 0.45
28	**Length (mm)**	9 ± 0.22	9 ± 0.22	9 ± 0.45	9 ± 0.45
	Weight (mg)	7 ± 0.22 [a]	6.5 ± 0.45 [a]	6 ± 0.45 [a]	5.5 ± 0.32 [b]
35	**Length (mm)**	12 ± 0.55	12 ± 0.55	11 ± 0.50	11 ± 0.50
	Weight (mg)	12 ± 0.55 [a]	11 ± 0.63 [a]	10 ± 0.50 [b]	9.5 ± 0.50 [b]
Survival %		25 [a]	25 [a]	20 [b]	19 [b]

Green water (GW); Clear water (CW); Rotifers (R); *Nannochloropsis oculata* (N); Yeast (Y).
Significantly different means ($P < 0.05$) are indicated by different letters (Tukey test).

Effects of *Artemia* feeding regime on growth in length, weight and survival rate of *Liza ramada* larvae (36 - 63 dayspost-hatching):

Results in Table (3) showed that, *Liza ramada* larvae at 36-63 days post-hatching, fed on different *Artemia* feeding regimes exhibited great variations in length, weight and survival rate. At the end of 63 days post-hatching, the highest average body length (22±1 mm), weight (72±1 mg) and survival rate (17.5%) in the larvae fed on GW+A enriched with N. Lowest values in the average body length (20±1.09 mm), body weight (65±1.84 mg) and survival rate (12%) were recorded in the larvae fed on CW+A enriched with Y (Figs. 5 - 7).

Table 3: Effects of *Artemia* nauplii (A) feeding regime, with or without *Nannochloropsis oculata* (N) and yeast (Y), on the length (mm), weight (mg) and survival rate (%) at 36 - 63 days post-hatching of *Liza ramada* larvae.

Days post-hatching		Feeding regimes *Artemia* nauplii (A) (for 28 days)			
		GW + A enriched with N	**GW + A enriched with Y**	**CW + A enriched with N**	**CW + A enriched with Y**
42	**Length (mm)**	15± 1.43 [a]	15.1± 1.39 [a]	14.1 ± 0.83 [b]	13.5± 0.67 [b]
	Weight (mg)	14 ± 1.26 [a]	13 ± 0.63 [a]	12 ± 1.26 [b]	11.5 ± 0.55 [b]
49	**Length (mm)**	16.1± 1.46 [a]	16 ± 1.28 [a]	15.1 ± 0.70 [b]	14.5 ± 0.45 [b]
	Weight (mg)	20 ± 1.26 [a]	20 ± 1.09 [a]	18.1± 0.70 [b]	17.4 ± 0.49 [b]
56	**Length (mm)**	16.3 ± 1.57	16.25± 0.60	16.05 ± 0.52	15.55 ± 0.41
	Weight (mg)	35 ± 0.63 [a]	34.2 ± 0.75 [a]	32 ± 1.48 [b]	30 ± 0.89 [c]
63	**Length (mm)**	22± 1 [a]	22 ± 1.34 [a]	21 ± 0.77 [a]	20 ± 1.09 [b]
	Weight (mg)	72 ± 1 [a]	70.2 ± 1.40 [b]	69± 1.34 [b]	65 ± 1.84 [c]
Survival %		17.5 [a]	17.5 [a]	13 [b]	12 [b]

Green water (GW); Clear water (CW); *Artemia* nauplii (A); *Nannochloropsis oculata* (N); Yeast (Y). Significantly different means (P < 0.05) are indicated by different letters (Tukey test).

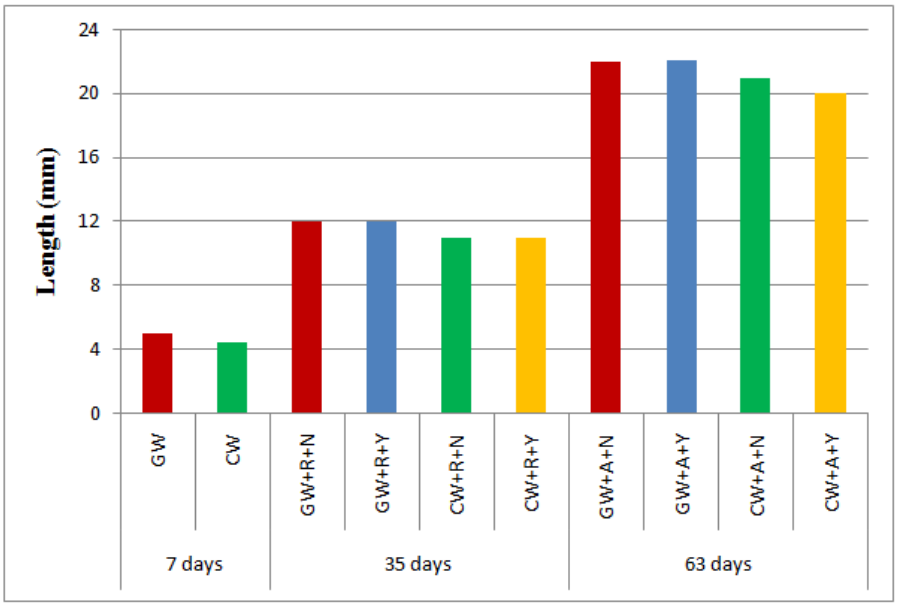

Fig. 5: Final total length (mm) of *Liza ramada* larvae fed on different feeding regimes at 7, 35 and 63 days post-hatching.

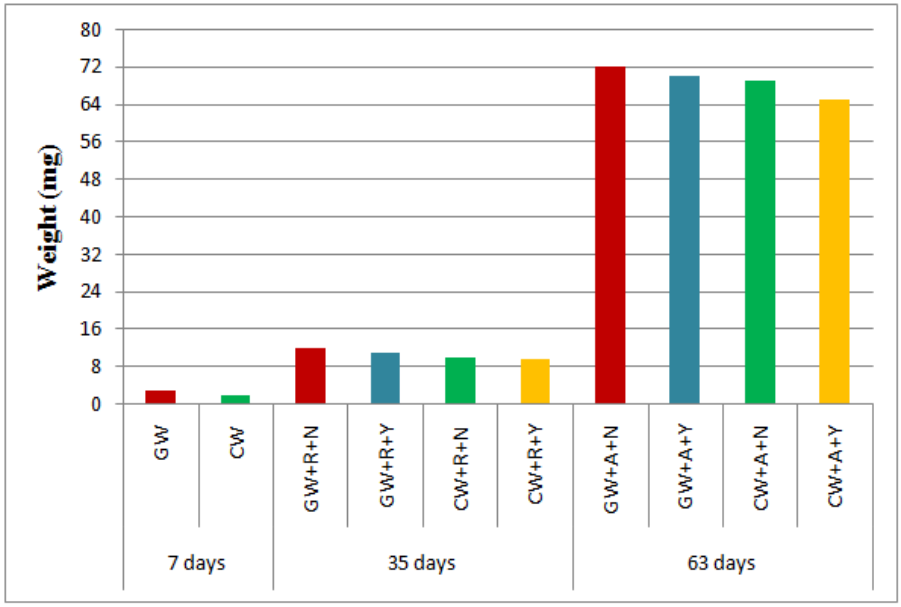

Fig. 6: Final total weight (mg) of *Liza ramada* larvae fed on different feeding regimes at 7, 35 and 63 days post-hatching.

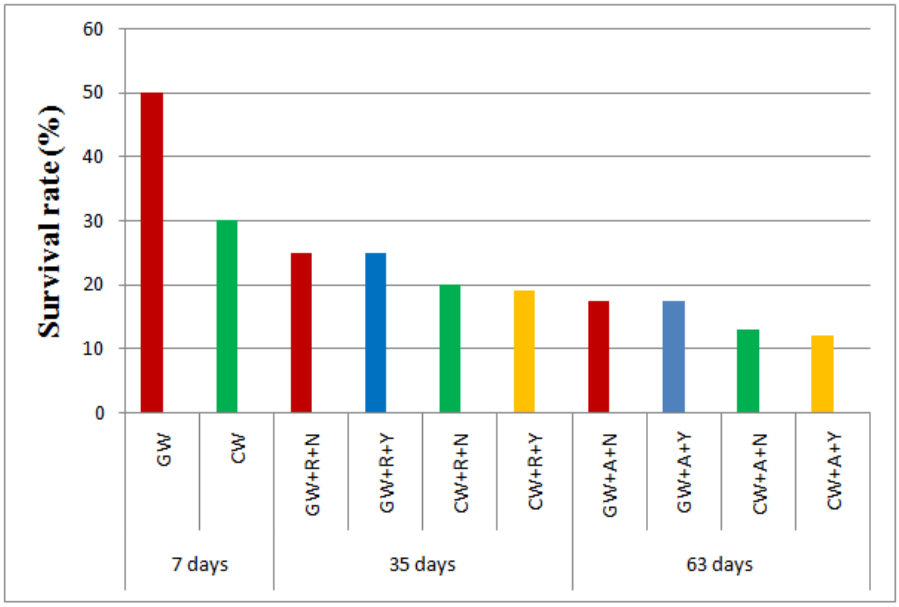

Fig. 7: Survival rate (%) of *Liza ramada* larvae fed on different feeding regimes at 7, 35 and 63 days post-hatching

DISCUSSION

In the present study, high rate (100%) of final oocyte maturation (FOM), ovulation and spawning was achieved in *L. ramada* females utilizing the pregnyl (HCG) as a priming injection at a dose of 20,000 IU/kg body weight followed, 24 h later, by resolving injection of 40,000 IU HCG/kg body weight. Similar observation was obtained by Mousa (2010). A variety of hormonal treatments was used for inducing final maturation and changeover of *M. cephalus* female with vitelline oocytes (tertiary yolk stage) to spawning condition with much higher doses: 50,000-80,000 IU HCG/kg body weight (Kuo *et al*., 1973); 28-48 mg of fresh mullet pituitaries plus 10,000-80,000 IU chorionic gonadotropin/kg of body weight

(Kulikova and Gnatchenko, 1987); and 20 mg carp pituitary homogenate/kg body weight of fish, followed by 200 µg LHRH-a/kg of fish (Lee *et al*., 1987 and Suzuki *et al*., 1991). The injection of piscine or mammalian gonadotropins (GtHs) has been used successfully to induce ovulation in a wide range of species (King and Pankhurst 2004; Wang *et al*., 2010 and Falahatkar*et al*., 2018).

In the present study, water salinity of 35‰ and a temperature of 21°C were suitable for induced spawning in *L. ramada*. The salinity and water temperature of this study were close to those have been reported by Mousa and Khalil (2013).They reported that temperature appears to have an influence on the spawning of *L. ramada*. Similar observations indicated that temperature plays an important role inreproductive status and spawning of some fish (The induced spawning of *L. ramada*allowed us to integrate the scanty data on the development of this species.

In agreement with the results obtained by Mousa (2010), newly hatched larvae of *L. ramada*had25 somites and one oil globule situated in the posterior part of the yolk. The mouth and anus opened on the 5th day post-hatching, and feeding began five days after hatching. However, Tung (1973) recorded 24 myotomes in the newly hatched larvae of *M. cephalus*. He added that feeding began three to five days after hatching. Furthermore, in*Chelon labrosus*, the mouth opening was occurred 6 days 8 h after spawning (Boglione *et al*., 1992).

Lipids and fatty acids are major sources of metabolic energy during the embryonic and pre-feeding larval stages in fish. At hatch, yolk-sac larvae have high levels of these energy sources, but they are dramatically reduced during the endogenous feeding stage (Evans *et al*., 2000). Thus, start feeding larvae require a live food that provides sufficient levels of these energy sources. Live feeds commonly used in marine finfish hatcheries, particularly rotifers *Brachionus* spp. and brine shrimp *Artemia* spp., are inadequate and variable in nutritional value. Therefore, it is critical to enrich these prey items with essential nutrients (e.g., fatty acids) before feeding them to the fish larvae (Hernandez-Cruz *et al*., 1999 and Sorgeloos *et al*., 2001). Previous studies have shown growth performance of larval red porgy is affected by levels of highly unsaturated fatty acids (HUFAs) docosahexaenoic acid (DHA, 22-6:n-3) and eicosapen-taenoic acid (EPA, 20:5n-3) provided in live prey (Hernandez-Cruz *et al*., 1999 and Roo *et al*., 2009). More recent studies have shown that levels of the n-6HUFA arachidonic acid (ARA, 20:4n-6) in live prey can also affect growth or stress resistance in marine fin-fish larvae (Carrier *et al*., 2011 and Watanabe *et al*., 2014). Numerous commercial media have been formulated to boost HUFA profiles and other essential nutrients in live prey. These include preserved microalgae concentrates, yeast and lipid powders and semi-moist pastes, and freeze-dried heterotrophically-grown microalgae. These products yield different nutritional biochemical profiles in live prey which markedly affect growth and stress resistance of the marine fish larvae fed enriched prey (Faulk and Holt, 2005; Garcia *et al*., 2008; Cavalin and Weirich, 2009).

In the present study, green water culture with *N. oculata* at 250 000 cells mL^{-1} resulted in higher *L. ramada*growth and survival compared with the clear water treatment. However, green water culture with high concentration of *N. oculata* (500 000 cells mL^{-1}) gave higher pigfish survival compared with the clear water treatment (Broach *et al*., 2017). Also, green water culture has improved growth and survival in larvae of many marine fish including: Atlantic halibut *Hippoglossus hippoglossus* (Naas *et al*., 1992), summer flounder *Paralichthys dentatus* (Bengston *et al*., 1999), rainbow smelt *Osmerus mordax* (Mitchill) (Ayer *et al*., 2005), cobia*Rachycentron canadum* (Faulk & Holt, 2005), and California yellowtail *Seriola lalandi* (Stuart and

Drawbridge, 2011). It is unknown whether this effect was the result of enhanced larval vision and increased feeding success, or direct or indirect nutritional advantages gained from algal additions to larval tanks. A review by Reitan *et al*. (1997) highlighted the ability of several marine finfish larvae to ingest algae, directly or indirectly, and assimilate algal nutrients. The improvement of length and weight of fish larvae fed on green water may be due to found of phytonutrients in micro algae, *Nannochloropsis oculata*.Howell *et al*. (1998) mentioned that feeding on algae during early developmental stages may provide the larvae with essential nutrients, may act as an initiator for the digestive system, or may have an effect on the micro flora of the larvae.

In the present studies, rotifer and *Artemia* enrichment with *N. oculata* significantly improved growth and survival of *L. ramada* larvae compared with larvae fed yeast-enriched rotifers and *Artemia*. Similarly, rotifer enrichment with *N. oculata* significantly improved survival of larval pigfish compared with larvae fed non-enriched rotifers (Broach *et al*., 2017). The improvement of length and weight of *L. ramada*larvae fed on GW+R enriched with N may be due to rotifer fed on micro algae, *Nannochloropsis oculata,* which complete benefits of nutrients. These results were matching with Watanabe *et al.*(2016). He mentioned that the larval performance of red porgy, *Pagrus pagrus* was improvement in growth and survival, when feeding on rotifer enriched by microalgae *N. oculata* or *Tetraselmis chuii*.In addition, Thépot *et al*.(2016) reported that the barramundi, *Lates calcarifer* larvae were fed on rotifers enriched with *N. oculata* and *Chlorella vulgaris* give better growth, faster development and higher stress resistance. Feeding rotifers with baker's yeast may provide adequate quantities of rotifers for fish larvae production. However, despite the fact that rotifers may synthesize de novo long chain PUFA (Lubzens *et al*., 1985), the low rate of synthesis results in accumulation of inadequate amounts of these essential fatty acids (EFA) in yeast-fed rotifers. It is well established that consequently these rotifers would not support normal development and survival of fish larvae (Koven *et al*., 1989 and Lubzens *et al*., 1995). Only rotifers which are raised on a limited number of unicellular algae will deliver the EFA to the fish larvae. Also, Brown *et al.* (1997) described that rotifers fed with microalgae (e.g. *Isochrysis* sp. and *N. oculata*) become rapidly enriched with ascorbic acid (AsA), whereas rotifers fed on baker's yeast (which itself is deficient in AsA) contained only residual amounts of AsA.; after 16 h of starving, rotifers lost ca. 10% of their AsA, while retaining ca. 50% of the total AsA ingested. Among the microalgae used to feed rotifers, the eustigmatophyte *Nannochloropsis* was found to support high rates of rotifer reproduction (Yamasaki *et al*., 1989; Ahmad, 1991 and Ferreira *et al*., 2018). This alga contains substantial quantities of EPA. It was also found to be suitable for enrichment of rotifers and *Artemia* with EFA, prior to using them for feeding the larvae (Watanabe, 1979; James and Abu Rezeq, 1989; Teshima *et al*., 1991; Lubzens *et al*., 1995 and Ferreira *et al*., 2018).

The improvement of length and weight of fish larvae fed on GW+A enriched with N may be due to feeding *Artemia* on micro algae, *N. oculata*, which complete benefits of nutrients (Watanabe *et al.*, 2016).Also, Merchie *et al*. (1995) noticed that the concentration of AsA in *Artemia* sp. may be increased by feeding with microalgae. In addition, Hafezieh *et al*. (2010) investigated that the effects of different *Artemia* enrichments containing variable amounts of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) on the growth and survival of larval Persian sturgeon (*Acipenser persicus*) and showed a positive effect of *Artemia* DHA proportions on growth and survival of the Persian sturgeon.

In conclusion, the presence of green algae *Nannochloropsis oculata*during *Liza ramada* larval rearing, in water or as enrichment media for live food, is essential not only for growth performance but also for raising survival rate. It is expected that the results of this study could guide future programs in hatchery technology and aquaculture of this commercially important species.

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ARABIC SUMMARY

تأثير إثراء الأغذية الحية على أداءالنمو ومعدل البقاء ليرقات أسماك الطوبار

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يعتمد نجاح تغذية يرقات الطوبار أثناء التطور المبكر على توفير البيئة والغذاء المناسب من حيث النوعية والكثافة. فى هذ البحث تمت دراسة تأثير إستخدام الروتيفرا المخصبة كغذاء أولى متبوعا بالأرتيميا المخصبة وأثر ذلك على النمو ومعدل البقاء لزريعة أسماك الطوبار. تم تحفيز التفريخ لأمهات أسماك الطوبار الناضجة للحصول على الزريعة.

تم تحضين الزريعة بعد الفقس فى أحواض زجاجية سعة ٥٠ لتر بكثافة ٢٠ يرقة/ لتر، تحت نظم تغذية مختلفة على ثلاث فترات زمنية؛ مياه خضراء تحتوى على طحلب النانوكلوربسس أكيولاتا بكثافة ٢.٥×١٠ ^{٥} خلية/ ملىلتر، روتيفرا ٨-١٠ كائن/ ملىلتر لمدة ٢٨ يوم،ثم أرتيميا ٤-٦ كائن/ ملىلتر لمدة ٢٨ يوم. تم إختبار أربع معاملات لكل من الروتيفرا والأرتيميا؛ مياه خضراء تحتوى على الطحالب مع الروتيفرا أو الأرتيميا والتى تم إثرائها بطحلب النانوكلوربسس أوكيولاتا أو الخميرة، مياه نظيفة بدون الطحلب مع الروتيفرا أو الأرتيميا والتى تم إثرائها بطحلب النانوكلوربسس أوكيولاتا أو الخميرة.

أوضحت النتائج تحسن فى معدلات النمو والبقاء ليرقات أسماك الطوبارة، عندما تغذت على مياه خضراء تحتوى على الطحالب مع الروتيفرا أو الأرتيميا والتى تم إثرائها بطحلب النانوكلوربسس أوكيولاتا. تم حساب معدل نموووبقاء اليرقات فى نهاية تجارب تحضين الزريعة عند اليوم السابع، اليوم الخامس والثلاثون واليوم الثالث والستون. تم الحصول على أعلى معدل نموووبقاء للزريعة المحضنة فى مياه تحتوى على طحلب النانوكلوربسس أكيولاتا. وتم تسجيل أقل معدل نموووبقاءلليرقات فى المياه النظيفة المغذاه بروتيفرا وأرتيميا مخصبة بالخميرة لمدة ٢٨ يوم لكل منهما. بينما تم الحصول على أعلى معدل نموووبقاءلليرقات فى المياه الخضراء المغذاه بروتيفرا وأرتيميا مخصبة بطحلب النانوكلوربسس أوكيولاتا لمدة ٢٨ يوم لكل منهما. وهذا يوضح أن طحلب النانوكلوربسس أكيولاتا ضرورى ليس فقط للتغذية الأولية لليرقات ولكن أيضا لتحسين جودة المياه. تغير وزن اليرقات تغيرا معنويا للمعاملات المختلفة. بينما لم يتغير طول اليرقات تغيرا معنويا لتلك المعاملات.

يمكن التوصية بأن وجود طحلب النانوكلوربسس أكيولاتا أثناء تحضين يرقات أسماك الطوبار سواء فى مياه التحضين أو كمادة لإثراء الغذاء الطبيعى يعتبر ضروريا ليس فقط لتحسين النمو ولكن أيضا لرفع معدل البقاء.