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Greenwater, Marine Bacillus subtilis HS1 Probiotic and Synbiotic Enriched Artemia and Rotifers Improved European Seabass Dicentrarchus labrax Larvae Early Weaning Length Growth, Survival, Water and Bacteriology quality

Salem Ahmed Md.[1, *], Nour A. M.[2], Srour T. M.[3], Assem S. S.[1], Ibrahim H. A.[4], El-Sayed H. S.[1]

[1]Fish Reproduction & Spawning Lab., Aquaculture Division, National Institute of Oceanography & Fisheries (NIOF), Quiet Bay Castle, El-Anfoshy, Alexandria, Egypt
[2]Animal and Fish Production Dep., Faculty of Agriculture (El-Shatby), Alexandria University
[3]Animal and Fish Production Dep., Faculty of Agriculture (Saba Basha), Alexandria University
[4]Marine Microbiology Lab., Marine Environment Division, (NIOF), Alexandria, Egypt

Email address:
ahmedmdsalem@yahoo.com (Ahmed Md. Salem)

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Abstract: The present study conducted in Fish Reproduction & Spawning Lab., NIOF, Alexandria, Egypt. European sea bass *Dicentrarchus labrax* newly hatched larvae from 8 dph to the 40 dph was reared according to (Süzer et *al*., 2011). The 6 tanks was green water with *N. salina* Algal count not less than 300000 cell/ml. The experimental treatments using green water using *N. salina* as positive control (G), green water plus marine probiotic bacteria (G+MP) and green water plus synbiotic (G+S).4 hours enriched rotifers *Brachionus plicatilis* started from the 7th dph until the 14th dph, the beginning of cofeeding on 6 hours enriched *Artemia fransiscana* (GSL) nauplii started and rotifers reduction started in the 18th dph and stopped from the 20th dph and artemia napulii fed alone until the 25th dph, 25dph larvae started feeding on artemia metanauplii and cofeeding with Orange® P 1/2 Small microdiets with 100-200 micron to 35dph. From the 35th dph artemia metanapulii stopped and larvae fed only on O.range® until the end of the experiment (the 40th dph). The treatments were green water using *Nanochlorapsis salina* algae (G), greenwater plus marine *Bacillus subtilis* HS1 Probiotic bacteria (G+MP) and greenwater plus synbiotic (G+S) in 30 l tanks in duplicates. Microbiological measurements were performed in water samples in every larvae critical stage (7, 14, 21, 25, 35 and 40dph) for colony forming unit (CFU) of total bacterial count, *Vibrio, Staphylococus, Aeromonas* and *Bacillus*. Also water quality measurements were performed in the beginning of the experiment in 2 pm and in the same time in the 7, 14, 21, 28, 35 and 40 dph newly hatched larvae tanks. Finally, the results of the 40dph early weaned larvae showed significantly ($p<0.05$) higher final total length achieved by (G+MP) followed by (G+S) and the lower significant ($p<0.05$) recorded by (G) treatment. The bacterial counts of the *Aeromonas* not detected in all treatments, the other bacterial counts showed promising results of (G+MP) and (G+S) than (G) treatments in both inhibiting potentially pathogenic bacteria counts and also in improving the potentially useful bacterial counts.

Keywords: Marine Probiotic, Synbiotic, Larvae, Early Weaning

1. Introduction

Egypt, the 8th world aquaculture producer is also the 1st Mediterranean Sea, Arab and African aquaculture producer (1). Egypt aquaculture production (986820 tons) divided to 61.88% tilapia, 11.55% mullets, 20.64% carps, 1.80% sea bass, 1.43% sea bream, 1.23% meager and 0.08% shrimps (2). Egypt marine aquaculture still depending on fry collected from natural resources (63 million fry) although Egypt had 6

hatcheries producing 15.8 million fry, only 1.6 million were sea bass fry (2). In 2013, Egypt mass mortalities in Kafr El-Sheik and El- Behira governorates tilapia fish farms which diagnosed as *A. hydrophilla* outbreak and also, in Maryut Valley, Alexandria governorate in European seabass, gilthead seabream and meagre farms but with different reasons of high temperatures and low oxygen stress in seabass and meagre, 100 tons equals 7 million L. E. loses (3, 4) and because of *Vibrio* sp. outbreak in gilthead seabream after seabass and seabream wild collected fry were transported to this farms (3).

2. Materials and Methods

The present study conducted in Fish Reproduction & Spawning Lab., NIOF, Alexandria, Egypt. European sea bass *Dicentrarchus labrax* newly hatched larvae from 8 dph to the 40 dph was reared according to (5**)**.

2.1. Fish Husbandry

The larval tanks was green water with *N. salina* Algal count not less than 300000 cell/ml. The experimental treatments using green water using *N. salina* as positive control (G), green water plus marine probiotic bacteria (G+MP) and green water plus synbiotic (G+S).4 hours enriched rotifers *Brachionus plicatilis* started from the 7th dph until the 14th dph, the beginning of cofeeding on 6 hours enriched *Artemia fransiscana* (GSL) nauplii started and rotifers reduction started in the 18th dph and stopped from the 20th dph and artemia napulii fed alone until the 25th dph, 25dph larvae started feeding on artemia metanauplii and cofeeding with O.Range® P 1/2 Small microdiets with 100-200 micron to 35dph. From the 35th dph artemia metanapulii stopped and larvae fed only on O.Range® until the end of the experiment (the 40th dph). The treatments were green water using *Nanochlorapsis salina* algae (G), greenwater plus marine *Bacillus subtilis* HS1 Probiotic bacteria (G+MP) and greenwater plus synbiotic (G+S) in 30 l tanks in duplicates.

2.2. Measurements of Length Growth

Growth of larvae were measured of total length (TL) to nearest 0.1 mm. were carried out using binocular light research microscope with graded eye piece. Fish larvae samples were measured at the beginning in 7dph also, at the end of the study in 40dph**)**, length growth parameters were performed. Fish survival rate were calculated according to (6) and average daily gain or increase in length were according to (7) and specific growth rate in length were according to (8).

2.3. Measurements of Water Quality

Water quality measurements were carried out biweekly using Hanna® HI9828 Water quality portable electric device were done in water quality in the beginning of the experiment in 2 pm and in the same time in the 7, 14, 21, 28, 35 and 40 dph newly hatched larvae tanks and the elctrolid device was genteelly putted in the bootom of the experimental tanks away from the air stone and water quality measurements are Dissolved Oxygen % (DO%), Dissolved Oxygen as mg/L (DO mg/L), pH, Salinity as part per thousand (ppt),Conductivity and Total Dissolved Solids (TDS).

2.4. Microbiological Measurements

2.4.1. Counting the Bacterial Groups

Microbiological measurements were performed in water samples in every larvae critical stages (7, 14, 21, 25, 35 and 40dph) for colony forming unit (CFU) of total bacterial count, *Vibrio, Staphylococus, Aeromonas* and *Bacillus* were done in the Microbiology Lab., Marine Environment Division, NIOF., Serial dilutions from 10^{-2} through 10^{-6} were made using filtered sterilized sea water. A portion (0.1 ml) from each appropriately diluted sample was used to inoculate plates prepared with seawater agar for total bacterial counting. Plates were incubated at 30^{o}C for 24 h. Plates of five selective media were inoculated with 1 ml of appropriately dilution sample for counting the different bacterial groups: total bacterial count, *Vibrio, Staphylococus, Aeromonas* and *Bacillus.*

2.4.2. Chemicals and Media

All chemicals used for biochemical tests and extraction of antimicrobial activity were of pure grade and purchased from Sigma chemicals, USA. Ingredients of media were all of analytical grade and obtained from recognized chemical suppliers (mainly Oxoid Co.). Media used throughout the work are described below. The composition is given in gl^{-1}. The pH value of the media was adjusted to 7.5 prior to sterilization. Autoclaving was occurred at 121 ^{o}C for 15 min.

Media Used for Isolation and Enumeration of the Different Bacterial Groups

a. Sea water agar

A selective medium by (9) used for determining total count bacteria. Peptone, 5; ferric phosphate, 0.1; agar, 15; sea water 1L.

b. Mannitol salt agar

A selective medium by (10) used for isolating *Staphylococcus* spp.: Peptone complex, 10; beef extract, 1; sodium chloride, 75; mannitol 10; phenol red, 0.025; agar 15.

c. Thiosulfate citrate bile salt sucrose agar (TCBS)

A selective medium by (11) used for isolating *Vibrio* spp.: Yeast extract, 5; peptone, 10; sodium thiosulfate, 10; sod-citrate, 10; Ox bile, 8; sucrose, 20; sodium chloride, 10; ferric chloride, 1; Bromothymol blue, 0.04; thymol blue, 0.04; agar, 14.

d. Aeromonas agar (Bile salts irgasan brilliant green agar (BSB) (LAB 167))

A selective medium for the isolation of *Aeromonas* spp: beef extract, 5; meat peptone, 5; xylose, 10; bile salts No. 3, 8.5; sodium thiosulphate, 5.44; irgasan, 0.005; brilliant green, 0.005; neutral red, 0.025; agar, 11.5.

2.5. Statistical Analysis

Statistical analysis were performed using analysis of variance (ANOVA), differences among means were considered significant at $p < 0.05$ multiple range of post hoc comparisons were performed using the least significant difference (LSD) to resolve the differences among the means of replication using basic statistics, STATISTICA® software for Windows (12).

3. Results

3.1. Growth and Survival

Finally, the results in Table 1 of the 40dph early weaned larvae showed significantly ($p<0.05$) higher final total length, total length gain, ADG and G% achieved by (G+MP) treatment while (G+MP) and (G+S) treatments showed significantly ($p<0.05$) higher larval SGR% and survival %.

Table 1. *Effect of using green water, green water plus marine Probiotic and green water plus Synbiotic treatments on European seabass (D. labrax) newly hatched larvae length growth parameters in mm.*

Parameter	G	G+MP	G+S
Initial TL (7DPH)	4.182 ± 0.195	4.272 ± 0.140	4.162 ± 0.063
Final TL (40DPH)	9.500^{c} ± 0.767	12.667^{a} ± 0.814	11.383^{b} ± 0.618
Total length gain	5.318^{c} ± 5.755	8.395^{a} ± 0.765	7.222^{b} ± 0.658
Total length ADG (mm/d)	0.161^{c} ±0.021	0.255^{a} ±0.023	0.219^{b} ±0.020
Total length SGR (%/d)	2.481^{b} ±0.223	3.290^{a} ±0.184	3.046^{a} ±0.199
Total length G%	127.291^{c} ±16.608	196.590^{a} ±17.155	173.693^{b} ±17.656
Final Survival %	7.592^{b} ± 3.949	18.407^{a} ± 1.152	20.111^{a} ± 2.768

Letters in the same row are for effects difference significance ($P < 0.05$).
G: Green water, G+MP: Green water plus marine probiotic; G+S: Green water plus synbiotic.
TL: Total length, ADG: Average daily gain in mm per day, SGR: Specific growth rate in % per day. G%: Gain %.

3.2. Water Quality

The results in Table 2 showed that the effect of using (G, G+MP and G+S) treatments on European seabass (*D. labrax*) 7, 14, 21, 28, 35 and 40 dph newly hatched larvae tanks dissolved oxygen % (DO%) in the 7dph tanks showed that the best significant ($P < 0.05$) results achieved by (G+MP) and (S). The 14, 35 and 40dph larvae tanks showed no significant differences while the best DO% resulted by (G+MP). The 21and 28dph larvae tanks showed no significant differences while the best DO% indicated by (G). The dissolved oxygen in mg/l (DO mg/l) in the 7dph larvae tanks showed no significant differences while the best DO mg/l achieved by (G+S). The 14, 35 and 40dph larval tanks showed no significant differences while the best DO mg/l resulted by (G+MP). The 21 and 28dph larvae tanks showed no significant differences while the best DO mg/l achieved by (G). The pH in the 7 and 14dph larvae tanks showed no significant differences while the best pH achieved by (G), (S) and (MP) with the same means. The 21and 28dph larvae tanks showed no significant differences while the best pH resulted by (G). The 35 and 40dph larvae tanks showed no significant differences while the best pH achieved by (MP) and (G). The salinity in mg/l (Sppt) in the 7dph larvae tanks showed that the biggest significant ($P < 0.05$) Sppt achieved by (G). The 14dph larvae tanks showed that the biggest significant ($P < 0.05$) Sppt recorded by (G+S). The 21, 28, 35 and 40dph larvae tanks showed no significant differences while the best Sppt achieved by (G).

The conductivity in mS/cm (Cond.) in the 7dph larvae tanks showed that the biggest significant ($P < 0.05$) Cond. achieved by (G). The 14dph larvae tanks showed that the biggest significant ($P < 0.05$) Cond. indicated by (G+S). The 21, 28 and 40dph larvae tanks showed no significant differences while the best Cond. achieved by (G). The 35dph larvae tanks showed no significant differences while the best Cond. exhibited by (G+S). The total dissolved solids (TDS) in ppm in the 7dph larvae tanks showed that the biggest significant ($P < 0.05$) TDS achieved by (G). The 14dph larvae tanks showed that the biggest significant ($P < 0.05$) TDS resulted by (G+S). The 21, 28, 35 and 40dph larvae tanks showed no significant differences while the best TDS achieved by (G). The Temperature in the 7, 14, 21, 35 and 40dph larvae tanks showed no significant differences while the biggest values achieved by (G). The 28dph larvae tanks showed no significant differences while the best result achieved by (G+S).

3.3. Bacterial Counts

The Total bacterial counts (TBC) were determined in three treatments (G, G+MP and G+S) treatments on newly hatched larvae tanks water using nutrient agar medium. Results in Table 3 presented that the TBC in the water source used in the 6 tanks stocking showed the same counts (1040 CFU/ml). The TBC in the pre stocking samples of 2dpf eggs showed the same results (1080 CFU/ml). The TBC in the samples of algae used as source of green water showed the same results (3100 CFU/ml). The TBC in the 7, 14, 21, 25, 35 and 40dph larvae tanks showed that the highest significant ($P < 0.05$) TBC achieved by (G). The *Aeromonas* counts (ABC) were determined in three treatments on newly hatched larvae tanks water using nutrient agar medium indicated that there was no ABC detected in all treatments. The *Staphylococcus* counts (SBC) were determined on newly hatched larvae tanks water using mannitol salt agar medium. The SBC in the pre stocking samples of 2dpf eggs showed the same counts (2050 CFU/ml).

The SBC in the samples of algae used as source of green water exhibited the same records (3100 CFU/ml). The SBC in the 7dph larvae tanks showed that the highest significant

($P < 0.05$) SBC achieved by (G). The SBC in the 14dph larvae tanks showed no significant difference while the highest SBC detected by (G+MP). The SBC in the 21dph larvae tanks showed no significant difference while the highest SBC achieved by (G).

Table 2. *Effect on European seabass (D. labrax) 7, 14, 21, 28, 35 and 40 dph newly hatched larval tanks water quality parameters.*

Par.	Age	G	G+MP	G+S
DO%	7dph	$93.95^{b} \pm 1.48$	$102.00^{a} \pm 0.00$	$102.00^{a} \pm 1.41$
	14dph	92.50 ± 2.12	99.00 ± 5.66	95.50 ± 0.71
	21dph	99.50 ± 10.61	94.00 ± 15.56	93.00 ± 2.83
	28dph	99.50 ± 6.36	99.00 ± 8.49	93.50 ± 7.78
	35dph	93.00 ± 9.90	93.50 ± 3.54	85.50 ± 0.71
	40dph	93.50 ± 9.19	100.50 ± 0.71	91.50 ± 2.12
DO mg/L	7dph	7.55 ± 0.07	8.05 ± 0.07	8.16 ± 0.32
	14dph	7.35 ± 0.07	7.78 ± 0.25	7.50 ± 0.28
	21dph	7.80 ± 0.85	7.25 ± 1.06	7.35 ± 0.21
	28dph	7.40 ± 0.57	7.40 ± 0.85	7.10 ± 0.42
	35dph	7.10 ± 0.85	7.20 ± 0.28	6.50 ± 0.14
	40dph	6.90 ± 0.71	7.60 ± 0.00	6.85 ± 0.07
pH	7dph	8.08 ± 0.00	8.08 ± 0.01	8.08 ± 0.01
	14dph	8.10 ± 0.00	8.10 ± 0.14	8.10 ± 0.00
	21dph	7.95 ± 0.07	7.90 ± 0.14	7.84 ± 0.06
	28dph	7.85 ± 0.07	7.80 ± 0.14	7.70 ± 0.00
	35dph	7.80 ± 0.14	7.80 ± 0.00	7.60 ± 0.00
	40dph	7.95 ± 0.21	7.95 ± 0.07	7.70 ± 0.00
Salinity mg/L	7dph	$37.77^{a} \pm 0.05$	$37.50^{b} \pm 0.00$	$37.54^{b} \pm 0.05$
	14dph	36.60 ± 0.00	$36.40^{b} \pm 0.14$	$36.80^{a} \pm 0.00$
	21dph	36.60 ± 0.14	36.30 ± 0.42	36.60 ± 0.00
	28dph	36.90 ± 0.14	36.60 ± 0.28	36.70 ± 0.00
	35dph	37.65 ± 0.21	37.30 ± 0.42	37.15 ± 0.07
	40dph	37.90 ± 0.14	37.65 ± 0.35	37.40 ± 0.00
Conductivity mS/cm	7dph	$56.75^{a} \pm 0.13$	$56.33^{b} \pm 0.04$	$56.48^{b} \pm 0.06$
	14dph	55.05 ± 0.07	$54.88^{b} \pm 0.17$	$55.30^{a} \pm 0.00$
	21dph	55.10 ± 0.14	54.55 ± 0.78	55.00 ± 0.00
	28dph	55.45 ± 0.21	55.10 ± 0.42	55.25 ± 0.07
	35dph	56.55 ± 0.21	56.10 ± 0.57	55.85 ± 0.07
	40dph	56.85 ± 0.21	56.50 ± 0.42	56.00 ± 0.00
TDS ppm	7dph	$28.38^{a} \pm 0.07$	$28.14^{b} \pm 0.06$	28.22 ± 0.01
	14dph	27.55 ± 0.07	$27.46^{b} \pm 0.11$	$27.70^{a} \pm 0.00$
	21dph	27.55 ± 0.07	27.40 ± 0.14	27.50 ± 0.00
	28dph	27.70 ± 0.14	27.55 ± 0.21	27.60 ± 0.00
	35dph	28.25 ± 0.07	28.00 ± 0.28	27.90 ± 0.00
	40dph	28.40 ± 0.14	28.20 ± 0.28	28.00 ± 0.00
Temperature °C	7dph	14.75 ± 0.07	14.70 ± 0.00	14.70 ± 0.00
	14dph	15.00 ± 0.00	15.00 ± 0.00	15.00 ± 0.00
	21dph	15.95 ± 0.07	15.85 ± 0.07	15.90 ± 0.00
	28dph	17.95 ± 0.07	17.75 ± 0.21	18.00 ± 0.00
	35dph	17.00 ± 0.00	16.85 ± 0.21	16.90 ± 0.00
	40dph	18.65 ± 0.07	18.55 ± 0.21	18.60 ± 0.00

Letters in the same row are for effects difference significance ($P < 0.05$).

The SBC in the 25dph larvae tanks showed no significant difference while the highest SBC exhibited by (G+MP). The SBC in the 35 and 40dph larvae tanks showed that the highest significant ($P < 0.05$) SBC resulted by (G) and (G+S). The *Vibrio* counts (VBC) were determined on newly hatched larvae tanks water using nutrient agar medium. The VBC in the pre stocking samples of 2dpf eggs showed the same counts (400 CFU/ml). The VBC in the samples of algae used as source of green water exhibited the same records (40 CFU/ml). The VBC in the 7 and 21dph larvae tanks showed no significant differences while the highest VBC achieved by (G+S). The VBC in the 14dph larvae tanks showed no significant difference while the highest VBC indicated by (G). The VBC in the 25dph larvae tanks showed no significant difference while the highest VBC resulted by (G+MP). The VBC in the 35dph larvae tanks showed that the highest significant ($P < 0.05$) VBC detected by (G+MP). The VBC in the 40dph larvae tanks showed that the highest significant ($P < 0.05$) VBC exhibited by (G). The *Bacillus* counts (BBC) were determined in three treatments on newly hatched larvae tanks water on nutrient agar medium in the pre stocking samples of 2dpf eggs showed the same counts (1925 CFU/ml). The BBC in the samples of algae used as source of green water exhibited the same records (2000 CFU/ml). The BBC in the 7, 14, 21 and 25dph larvae tanks showed that the highest significant ($P < 0.05$) BBC achieved by (G+MP). The BBC in the 35 and 40dph larvae tanks showed that the highest significant ($P < 0.05$) BBC exhibited by (MP) and (S).

4. Discussion

The present study indicated that the results of using green water, green water plus marine *B. subtilis* probiotic and green water plus synbiotic on larvae length gains showed that better significantly results achieved by G+MP and G+S than G in most larvae body length and length gains parameters of 40dph, showed potential application of them in these critical stages and these may be due to many factors and mechanisms such as positive effects of these treatments as supported by (13) who showed enhanced growth of gilthead seabream larvae using *Bacillus* sp. bacteria. These results supported by (14) who reviewed that the *Bacillus* mixture used in his study has been evaluated before as a candidate probiotic mixture, mainly for shrimp but also in fish larviculture. In those experiments, increased growth rates of shrimp and fish larvae and reduced *Vibrio* levels were obtained. *B. subtilis* and *B. licheniformis* were found to increase resistance to pathogenic *Yersinia ruckeri* in rainbow trout. In gilthead seabream, an improved cellular innate immune response was found when *B. subtilis* and *L. delbrueckii* were given. Reference (15) showed significant growth, SGR and survival of *S. aurata* larvae treated with probiotic. Larvae husbandry enhanced by Probiotic supplemented live food feeding. Significant results achieved by probiotic treated live food and live foods due to of *Lactobacillus* bacteria significant proportion changing in the gut flora the significant. This in agreement with (16, 17) studies on Indian white shrimp, *F. indicus*, and shrimp *P. vannamei*. Probiotics treated live food had more effective digestive enzymes due to bacterial colonization in the larval gut (15). Also suggested that the growth, husbandry and digestive process enhanced by probiotics through several beneficial microbial balance and specific enzymes activities mechanisms. This were in agreement with (18) study that recorded increased *D. labrax* larvae survival, growth, digestive enzyme activities when treated with live yeast *Debaryomyces hansenii*.

European sea bass newly hatched larvae results, under high intial stocking densities of 50 fertilized egg per ml, putting in considerations recent industerial larvae rearing protocols in the developed marine hatcheries had significant survival rates of using G+S and G+MP compared to G treatment in agreement with (19) whom reviewed that the development of an aquaculture industry relies on the profitability of its culture protocols. The methods utilized to raise a species not only have to be reliable and highly productive, as they have to be relatively inexpensive (19). This imposes several challenges to producers since a culture protocol that guaranties higher survival is not necessarily the most productive or profitable (19). For instance, higher stocking densities may generate lower survival, but be more productive than lower stocking densities (19). One of the greatest expenses for an aquaculture facility is the feeds, particularly the ones destined to the larvae (19).

Reference (20) indicated that the weaning period from *Artemia* to formulated feed decline in growth could also be partly explained by the hardness or the taste of the formulated feed or its lower palatability compared to high water content thin exoskeleton live foods. Reference (21) indicated that formulated feed not being preferred by gilthead seabream at the start of the co-feeding period. Reference (22) reported that some conducted studies have evaluated synbiotics impacts on survival, growth parameters or feed utilization. Rainbow trout treated for 12 weeks using *E. faecalis* and MOS/PHB had no survival rate differences (23). The Japanese flounder fed *B. clausii* and MOS/FOS was healthy and showed high survival, active ingestion and better growth (24). Reference (25) assessed commercial synbiotic, Biomin IMBO, (probiotic (*E. faecium* $5x10^{11}$ CFU/kg) and FOS as prebiotic), in three levels 0.5, 1.0 and 1.5 g per kg of commercial rainbow trout food. However, synbiotic treated rainbow trout fingerlings (25) showed significantly higher survival rate than control. The highest average of survival rate was observed in the 1 g per kg feed that was statistically different from lower, higher doses and the control groups. In *B. subtillis*/FOS or *B. subtillis*/chitosan synbiotics treated yellow croaker and cobia showed no survival rate differences (26, 27).

Table 3. *Effect of using green water (G), green water plus marine Probiotic (MP) and green water plus Synbiotic (S) treatments on European seabass (D. labrax) newly hatched larvae bacterial counts (CFU/ml).*

Parameter		G	G+MP	G+S
Total bacterial counts (CFU/ml)	Water source	1040.00 ± 113.14	1040.00 ± 113.14	1040.00 ± 113.14
	Algae	3100.00 ± 141.42	3100.00 ± 141.42	3100.00 ± 141.42
	7dph	1280.00^{a} ± 113.14	768.00 ± 271.53	440.00^{b} ± 56.57
	14dph	720.00 ± 905.10	480.00 ± 226.27	632.00 ± 803.27
	21dph	560.00 ± 113.14	460.00 ± 28.28	520.00 ± 282.84
	25dph	640.00 ± 0.00	560.00 ± 113.14	500.00 ± 424.26
	35dph	760.00^{a} ± 56.57	340.00^{b} ± 28.28	380.00^{b} ± 28.28
	40dph	880.00^{b} ± 113.14	1280.00^{a} ± 113.14	1550.00^{a} ± 70.71
Staphylococcus count (CFU/ml)	Water source	690.00 ± 268.70	690.00 ± 268.70	690.00 ± 268.70
	Algae	3100.00 ± 141.42	3100.00 ± 141.42	3100.00 ± 141.42
	7dph	1025.00^{a} ± 35.36	1020.00^{a} ± 28.28	400.00^{b} ± 113.14
	14dph	180.00 ± 28.28	420.00 ± 28.28	280.00 ± 169.71
	21dph	700.00 ± 28.28	430.00 ± 98.99	485.00 ± 162.63
	25dph	480.00 ± 56.57	560.00 ± 226.27	380.00 ± 28.28
	35dph	1160.00^{a} ± 56.57	500.00^{b} ± 84.85	940.00^{a} ± 84.85
	40dph	640.00^{a} ± 56.57	420.00^{b} ± 28.28	660.00^{a} ± 84.85
Vibrio count (CFU/ml)	Water source	17.50 ± 3.54	17.50 ± 3.54	17.50 ± 3.54
	Algae	40.00 ± 0.00	40.00 ± 0.00	40.00 ± 0.00
	7dph	30.00 ± 14.14	40.00 ± 21.21	45.00 ± 21.21
	14dph	71.00 ± 97.58	2.00 ± 0.00	48.50 ± 58.69
	21dph	90.00 ± 42.43	60.00 ± 28.28	120.00 ± 113.14
	25dph	85.00 ± 49.50	170.00 ± 14.14	105.00 ± 77.78
	35dph	140.00^{b} ± 14.14	420.00^{a} ± 226.27	155.00^{b} ± 7.07
	40dph	255.00^{a} ± 7.07	77.50^{b} ± 31.82	70.00^{b} ± 21.21
Bacillus count (CFU/ml)	Water source	5.50 ± 0.71	5.50 ± 0.71	5.50 ± 0.71
	Algae	2000.00 ± 0.00	2000.00 ± 0.00	2000.00 ± 0.00
	7dph	24.00^{b} ± 1.41	1625.00^{a} ± 247.49	11.50^{b} ± 9.19
	14dph	5.00^{b} ± 1.41	2225.00^{a} ± 35.36	19.00^{b} ± 1.41
	21dph	6.00^{b} ± 1.41	2900.00^{a} ± 141.42	31.00^{b} ± 26.87
	25dph	7.00^{b} ± 2.83	2550.00^{a} ± 70.71	9.50^{b} ± 2.12
	35dph	8.00^{b} ± 4.24	1300.00^{a} ± 141.42	800.00^{a} ± 282.84
	40dph	42.50^{b} ± 10.61	1900.00^{a} ± 141.42	2050.00^{a} ± 70.71

Letters in the same row are for effects difference significance ($P < 0.05$).
G: Green water, G+MP: Green water plus marine probiotic; G+S: Green water plus synbiotic.

The present study water quality results performances were within suitable limits for larval rearing tanks in agreement with (8, 28). Reference (29) showed that probiotics could significantly reduce the concentrations of nitrogen and phosphorus in pond water compared with the control in shrimp, *P. vannamei* ponds and the use of *B. coagulans* SC8168 in shrimp larvae as water additive had shown inconsistent results. In accordance with present obtained results (30) showed that there was no obvious effect of probiotic on the water quality. Newly isolated strains of *B. subtilis*, *B. cereus* and *B. licheniformis* were selected and evaluated as potential biological agents for the enhancement of the water quality in cultures of ornamental fish, and it was found that the selected isolates reduced together synergistically the level of pathogens and the concentrations of waste ions *in vitro* and *in vivo* and therefore, the strains were considered to be safe for use in ornamental aquaculture (31).

The bacterial counts of the potentially pathogenic bacteria such as *Aeromonas sp., Staphylococcus sp.* and *Vibrio sp*., or as potentially useful bacteria such as *Bacillus sp* and total bacterial count in the three treatments showed that the potentially pathogenic bacteria reduced by MP followed by S than G similarly with (32) concluded that the bacteriocin-like compound produced by *B. subtilis* SH1 possesses an antimicrobial activity against a number of Gram-positive and Gram-negative bacteria, yeasts and fungi. The inhibitory effect of the compound against human pathogens such as *S. aureus* ATTC 6538 and *P. aeruginosa* ATTC 8739 suggests promising applications in the clinical field. The antifungal activity of the compound against some tested plant pathogens supports the utilization of *B. subtilis* SH1 as a good candidate for crop protection. (14) who reviewed that by adding a *Bacillus* mixture, larger changes in the bacterial communities between the initial and final experiment were observed. This implied that, by the end of the experiment, the *Bacillus* mixture probably was not able to maintain their initial composition. The subtle difference of cell wall composition of the two types of yeast strain, resulted in a nearly 40% difference in bacterial community similarity at the end of the experiment. The two types of yeast strains induced divergent change in the distribution of bacterial species. These results indicate changes in the composition and the evenness in a MC can be directed by the inputs of probiotics or different yeast strains. These changes might have an impact on the development of the intestinal microbiota of the fish larvae. Farmers often consider the probiotic as a replacement of chemicals and antibiotics, and as a result misused them as drugs and expected to see instant effects on the fish. Reference (33) reported that several authors indicated that probiotics effects and actions on enhancement of nutrition of aquacultured host through digestive enzymes production and better growth and feed efficiency, intestinal disorders prevention and feeds ingredients antinutritional factors pre-digestion. Reference (34) showed that probiotics germinate in the intestine, grow and produce digestive enzymes using carbohydrates. (14) reported that probiotics can be administered either as a food supplement or as an additive to the water. Probiotics increased *P. vannamei* and *F. indicus* shrimps growth performance (17). References (15, 35) indicated that the probiotics enhanced the immune responses and bacterial loading in aquatic organisms and environments. Probiotics treatments by live food and/or culture water decreased bacterial activity in *S. aurata, P. dentatus*, *Scophthalmus maximus* (15, 36, 37).

Reference (14) showed that *in vivo* evaluations have proven that the *Bacillus* mixture has no deleterious effects on turbot. Furthermore, our experiments also confirmed the positive effects of the *Bacillus* mixture in terms of survival rate and reduction of *Vibrio* numbers, although the differences were not always significant. Yeasts, which are traditionally used as feed additives, have been promoted and used as probiotics and prebiotics in aquaculture (38). The yeast mutant had a positive effect (improved growth and protection against pathogen vibrios) on both rotifers and *Artemia*, under both gnotobiotic and open culture conditions. However, it was not known so far whether they have a beneficial or deleterious effect on fish larvae but showed a slight positive effect on the survival of turbot larvae, however the reduction on vibrios was not significant (14). Reference (14) reported that the socalled "microbial maturation of water" that delayed gut colonisation while increasing the survival rate of turbot larvae. An artificial enrichment of the flora with a lactic acid bacterium improved also the resistance of the larvae against a pathogenic *vibrio*. Reference (23) carried out for first time a challenge test in fish feed with synbiotics during 12 weeks. After 14 dpi with *V. anguillarum* all groups showed lower mortality than that control group, being significant in EM and EMP groups. Dietary *B. subtilis* supplementation in yellow croaker (26) during 10 weeks elevated the resistance to *V. harveyi* infection, but this response was not related with presence or concentration of FOS. No significant interactions were observed between dietary *B. subtilis* and FOS in the cumulative mortality after challenge. In (27) cobia fed diets supplemented with various levels of probiotic and chitosan for 56 days were challenged with *V. harveyi*. Post-challenge survival of fish was always significantly higher in high chitosan groups and increased at each chitosan level with the increase of *B. subtilis* supplementation level, which speculated chitosan and *B. subtilis* have a synergistic effect.

The obtained results of using green water algae, green water plus marine probiotic and green water plus synbiotic, which proved positive treatments could be explained by some factors in the contents of microalgae, the green water larval rearing conditioners and quality enrichment, also could be according to greenwater potentially useful bacteria which already colonized in the rearing water and adding marine bacilli probiotic or synbiotic containing multi bacilli and lactobacilli bacterial probiotic and bakery yeast fungal

probiotic, multi prebiotics and enzymes, so the treatments plus green water are added value to the larval rearing and early weaning. These results in agreement of (13) who showed enhanced growth of gilthead sea bream larvae using *Bacillus* sp. bacteria and (39) who showed that growth hormone (GH) concentrations in both pituitary gland and serum were low in vitamin B6-depleted rates. Reference (40) studied the effect of water treatment systems on gut microbial community (MC) in reared larvae of Atlantic cod and showed significant differences between water treatments on microbial communities supported. It is indicated that the process of stabilizing the microbial community of the rearing water results in enhancing larval growth and survival. (40) reported that early exposure to high bacterial densities may be important for immune tolerance. The zebra fish, it has been shown that the gut microbiota is necessary for the development of the immune system. Thus the establishment of a protective intestinal microflora will increase survival and growth of the fish larvae. Hence, the quality as well as quantity of early phase of several marine fish species highly depends on knowledge and possibility to control the complex interactions between the cultured organisms and the bacterial communities which develop at the mucosal surfaces, in the surrounding water, and the rearing systems. During the larvae stage, ingestion of bacteria may present antigens and be an important basis for the formation and development the immune system (40). This may result either in antigen priming or in development of immune tolerance to specific bacterial strains. These bacterial strains consist of aerobic, facultative anaerobic and obligate anaerobic forms and they are the principal colonizers in the GI tract of fish (40).

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